

65798

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
09/674195

INTERNATIONAL APPLICATION NO: PCT/US99/09151	INTERNATIONAL FILING DATE 27 April 1999	PRIORITY DATE CLAIMED 30 April 1998
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TITLE OF INVENTION NUCLEIC ACIDS OF THE M ANTIGEN GENE OF HISTOPLASMA CAPSULATUM, ANTIGENS VACCINES AND ANTIBODIES, METHODS AND KITS FOR DETECTING HISTOPLASMAS

APPLICANT(S) FOR DO/EO/US

THE GOVERNMENT OF THE UNITED STATES OF AMERICA...

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. has been transmitted by the International Bureau.
 - c. is not required, as the application was filed in the United States Receiving Office (RO/US).
6. A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. have been transmitted by the International Bureau.
 - c. have not been made; however, the time limit for making such amendments has NOT expired.
 - d. have not been made and will not be made.
8. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (unexecuted)
10. A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

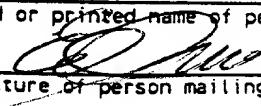
Items 11. to 16. below concern document(s) or information included:

11. An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. A **FIRST** preliminary amendment.
- A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. A substitute specification.
15. A change of power of attorney and/or address letter.
16. Other items or information:
 - General Authorization for Petition for Extension of Time
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U.S. APPLICATION NO. (1) 09/674,195

INTERNATIONAL APPLICATION NO
PCT/US99/09151ATTORNEY'S DOCKET NUMBER
65798

17. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):

Search Report has been prepared by the EPO or JPO	\$ 860.00
International preliminary examination fee paid to USPTO (37 CFR 1.482)	\$ 690.00
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))	\$ 710.00
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO	\$ 1000.00
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)	\$ 100.00

CALCULATIONS PTO USE ONLY**ENTER APPROPRIATE BASIC FEE AMOUNT =**

\$ 710.00

Surcharge of \$130.00 for furnishing the oath or declaration later than 20 30 months from the earliest claimed priority date (37 CFR 1.492(e)).

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CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	44 - 20 =	24	X \$ 18.00	\$ 432.00
Independent claims	7 - 3 =	4	X \$ 80.00	\$ 320.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$ 270.00	\$

TOTAL OF ABOVE CALCULATIONS =

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Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).

\$

SUBTOTAL =

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Processing fee of \$130.00 for furnishing the English translation later than 20 30 months from the earliest claimed priority date (37 CFR 1.492(f)).

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+

TOTAL NATIONAL FEE =

\$1462.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property

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charged	\$

a. A check in the amount of \$ 1462.00 to cover the above fees is enclosed.

b. Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-1135. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO.

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SIGNATURE:

Richard A. Kaba

NAME

30,562

REGISTRATION NUMBER

09/674195
526 Rec'd PCT/US 26 OCT 2000
PATENT APPLICATION
Attorney Docket No. 65798

PATENT COOPERATION TREATY
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(US/RO)

International
Application No.: PCT/US99/09151

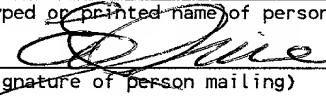
International
Filing Date: 27 April 1999

Priority Date: 30 April 1998

Applicant: THE GOVERNMENT OF THE
UNITED STATES OF AMERICA,
as represented by THE
SECRETARY OF THE DEPARTMENT
OF HEALTH AND HUMAN SERVICES

Title: NUCLEIC ACIDS OF THE M ANTIGEN
GENE OF *HISTOPLASMA CAPSULATUM*,
ANTIGENS, VACCINES AND ANTIBODIES,
METHODS AND KITS FOR DETECTING
HISTOPLASMAS

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PRELIMINARY AMENDMENT

Box PCT
Commissioner of Patents and Trademarks
Attention: Assistant Commissioner for Patents
Washington, D.C. 20231
Attn: RO/US

Dear Sir:

This Preliminary Amendment is submitted herewith for consideration of the above-identified national filing of the International application. Applicants respectfully request that the following new claims be considered in entering the national stage of the above-identified international application:

IN THE CLAIMS:

41. An isolated *Histoplasma Capsulatum* M antigen comprising a mature polypeptide consisting essentially of the polypeptide of SEQ ID NO:2 starting with the serine residue of amino acid position 17.

42. The isolated *Histoplasma Capsulatum M* antigen of claim 41, wherein the polypeptide is a recombinant polypeptide.

43. The isolated *Histoplasma Capsulatum M* antigen of claim 42, wherein the polypeptide is produced in a procaryotic host.

44. The isolated *Histoplasma Capsulatum M* antigen of claim 41, wherein the protein does not contain carbohydrate moieties.

Respectfully submitted,

FITCH, EVEN, TABIN & FLANNERY

By:


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09/674195

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NUCLEIC ACIDS OF THE M ANTIGEN GENE OF
HISTOPLASMA CAPSULATUM, ISOLATED AND RECOMBINANTLY-
PRODUCED ANTIGENS, VACCINES AND ANTIBODIES,
METHODS AND KITS FOR DETECTING HISTOPLASMOSIS

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BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates to reagents and methods for the detection of histoplasmosis. In particular, the present invention relates to nucleic acids (DNAs) relating to the M antigen gene of *Histoplasma capsulatum*; to vectors and host expression systems containing these nucleic acids; to nucleic acids (RNAs) which encode the M antigen of *H. capsulatum*; to isolated and recombinantly-produced antigens encoded by these nucleic acids; to antibodies produced against these antigens; to methods and kits for detecting histoplasmosis using these nucleic acids, antigens and antibodies; and to vaccines for the treatment or prevention of histoplasmosis.

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BACKGROUND

Histoplasmosis is a systemic fungal disease resulting from the inhalation or, less frequently, the ingestion of spores of the fungus *Histoplasma capsulatum*, variety *capsulatum*, which is worldwide in distribution.

25

The infection often causes acute pneumonia, or disseminated reticuloendothelial hyperplasia, or an influenza-like illness with joint effusion and erythema nodosum. Reactivated infection involves the lungs, meninges, heart, peritoneum and adrenals. Clinically

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inapparent or mild disease can result from limited, primary site infection of *H. capsulatum* in the lungs, but an often life-threatening, disseminated form of histoplasmosis can occur in immunodeficient patients, particularly the elderly, and those who have acquired immunodeficiency syndrome (AIDS). It is important to

properly identify *H. capsulatum* from other fungal species in order to determine the proper treatment for a fungal infection.

H. capsulatum is a dangerous, dimorphic, pathogenic fungus which, under different environmental conditions, may exist as either the yeast or mold phase. The organism exists as a multicellular mycelium at room temperature in rich soils, and in organic matter, in temperate environments worldwide, and proliferates as a unicellular yeast form at 37°C, and in infected host tissues. Only the yeast phase is known to survive within tissues, or within macrophages. The unicellular yeast form reproduces by budding on specialized media at 37°C. The mold form produces multicellular filamentous colonies that consist of cylindrical tubular structures called hyphae, and may contain microconidia and macroconidia which primarily grow under appropriate soil conditions, or on specialized fungal media, at 25°C. *H. capsulatum* occurs throughout the world, particularly in Brazil, Africa, India, Southeast Asia and the United States, but is most commonly found in soil from the fertile river valleys (Mississippi and Missouri river valleys) of the central United States.

H. capsulatum is associated with bird (particularly black bird and seagull) and bat excrement. (See, for example, Loyd et al., *Histoplasma capsulatum*. In Principles and Practice of Infectious Disease (3rd ed., Coordinating ed., Mandell et al., New York, (1990)); Wheat, "Diagnosis and Management of Histoplasmosis," Eur. J. Clin. Microbiol. Infect. Dis. 8:480 (1989).) The fungus infects the soil, and the resulting infected soil is often used as a habitat by birds and/or bats.

In addition to *H. capsulatum* var. *capsulatum*, two variants of *Histoplasma* exist: *H. capsulatum* var. *duboisii* (African histoplasmosis) and *H. capsulatum* var. *farciminosum* (epizootic lymphangitis of horses and

mules). (See, for example, Rippon, Histoplasmosis. In Medical Mycology The Pathogenic Fungi and the Pathogenic Actinomycets (3rd ed., Saunders Company, Chapter 15 (1988)).) Many strains of *H. capsulatum* are currently deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD, 20852. *H. capsulatum* strain CDC6623, deposited under accession number ATCC 26320, is discussed in Pine et al., "Procedures for the Production and Separation of H and M Antigens in Histoplasmin, and Chemical and Serological Properties of the Isolated Products," *Mycopathlogia* 61:131-141 (1977). The following other strains or variants of *H. capsulatum* are also deposited with the ATCC: *H. capsulatum* (attenuated Downs strain, filamentous phase, accession number ATCC 38904), *H. capsulatum* (attenuated Downs strain, yeast phase, accession number ATCC 38904), *H. capsulatum* (filamentous phase, accession number ATCC 11407), *H. capsulatum* (yeast phase, accession number ATCC 11407), *H. capsulatum* variant *duboisi* (filamentous phase, accession number ATCC 32281), *H. capsulatum* variant *duboisi* (yeast phase, accession number ATCC 32281), *H. capsulatum* variant *farciminosum* (filamentous phase, accession number ATCC 58332) and *H. capsulatum* variant *farciminasum* (yeast phase, accession number ATCC 58332).

The M antigen of *H. capsulatum* is a pluripotent glycoprotein having a molecular mass of 94 kDa, an isoelectric point of 4.7, oligosaccharide side chains, glycosidic epitopes which are N-linked to the peptide core, and protein epitopes, which have been shown to be unique to the *H. capsulatum* fungus. The peptide epitopes react with human antibodies, are not affected by N-deglycosylation, and trigger the proliferation of T cells. The M antigen is an immunodominant antigen of *H. capsulatum*, and elicits both humoral and cell-mediated immune responses. The glycopeptide bonds present in the glycoprotein are N linked. The M antigen of *H.*

capsulatum is considered to be the immunodominant antigen of *H. capsulatum* because antibodies generated against the M antigen are first to arise in infection, and are more commonly present during all phases of histoplasmosis.

5 Because the presence of this M antigen is indicative of histoplasmosis infection, the M antigen can serve as a marker for histoplasmosis infection. However, the biological identity of the M antigen has remained unknown. One report demonstrated that M protein was a
10 catalase, based upon its ability to react with anti-catalase antibodies.

Currently, histoplasmosis is diagnosed by culture, or by the demonstration of a rise in complement-fixing antibody titers in serum. A definitive diagnosis of an *H. capsulatum* infection currently requires the isolation and propagation of the fungus, which is time-consuming and lacking in sensitivity, and which is dangerous for laboratory personnel, who must take extreme caution to prevent inhalation of the pathogenic fungus, so as not to become ill with a pulmonary infection. Further, only small quantities of antigens of *H. capsulatum* for use as biological reagents may be prepared in this manner.

Conventional laboratory identification methods used to isolate and identify *H. capsulatum* include the culture of a clinical specimen at room temperature on specialized fungal media. This procedure isolates the slower growing *H. capsulatum* colonies from possible contaminants, such as bacteria, and from faster growing saprobic fungi.

This method, however, has several disadvantages. Because the growth of *H. capsulatum* to a visible colony normally takes from about two to four weeks, and sometimes as long as 12 weeks, this procedure is very slow. (See, for example, Rippon, Histoplasmosis. In Medical Mycology, The Pathogenic Fungi and the Pathogenic Actinomycetes, supra.; Koneman et al., Laboratory Identification of Molds, in Practical Laboratory Mycology, (3rd ed.

Williams & Wilkins (1985)); and McGinnis, *Histoplasma capsulatum*. In Laboratory Handbook of Medical Mycology (Academic Press (1986)).) Further, additional growth is required before the characteristic colony morphology and
5 microscopic sporulation pattern with tuberculate macroconidia may be observed. In addition, approximately 10% of cultures produce only smooth-walled macroconidia, and some cultures fail to sporulate. Moreover, many species of fungi other than *H. capsulatum*, such as
10 *Blastomyces dermatitidis*, *Chrysosporium* sp., and *Sepedonium* sp., produce similar colony and sporulation characteristics. Thus, additional testing is usually necessary to definitively identify the organism.

One method of converting the mycelial colony of *H. capsulatum* to the yeast phase is performed by subculturing the organism onto highly enriched cysteine-containing media, and incubating it at 35°-37°C. However, conversion to the yeast phase is often difficult, and may require several additional subcultures at three-day intervals.
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Serologic evidence is the prime diagnostic indicator of histoplasmosis. Such evidence may be obtained with several serologic tests, such as the immunodiffusion test, which detects precipitants against the species-specific H and M antigens found in histoplasmin. (See, for example, Kaufman, "Laboratory Methods for the Diagnosis and Confirmation of Systemic Mycoses," *Clin. Infect. Dis.* 14:23-29 (1992), and Wheat, "Diagnosis and Management of Histoplasmosis," *supra*.)
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30 Histoplasmin, an unpurified culture supernatant obtained from the mycelial phase of *H. capsulatum* grown in a chemically-defined medium containing *H. capsulatum* M antigens is currently used to probe both humoral and cell-mediated responses in patients with histoplasmosis.
35 It is used for the serologic diagnosis of histoplasmosis, and as a skin test antigen to demonstrate delayed

hypersensitivity to infection in skin tests for histoplasmosis. The purification of histoplasmin is described by Bradley et al, "Purification, Composition, and Serological Characterization of Histoplasmin-H and M Antigens," *Infect. Immun.* 9:870-880 (1974). The preparation of H and M antigens of *H. capsulatum* free of heterologous antigens is described by Green et al., "Preparation of h and m Antigens of *Histoplasma capsulatum* Free of Heterologous Antigens," *Curr. Microbiol.* 12:209-216 (1985). (See also, Pine, "Histoplasma antigens: their Production, Purification and Uses," *Contrib. Microbiol. Immunol.* 3:138-168 (1977).) The preparation of antisera to the M antigen is described by Green et al., "H and M Antigens of *Histoplasma capsulatum*: Preparation of Antisera and Location of these Antigens in Yeast-Phase Cells," *Infect. Immun.* 14:826-831 (1976). General information concerning the serodiagnosis of fungal diseases is present in L. Kaufman et al., Serodiagnosis of Fungal Diseases, in Manual of Clinical Laboratory Immunology (3rd ed., American Society for Microbiology, Washington, D.C. (1988)).

Although the M antigen of *H. capsulatum* is useful in immunoassays for the diagnosis of histoplasmosis, purification of the M antigen from a batch culture is a laborious and low-yield process. The use of a recombinantly-produced M antigen of *H. capsulatum* in such immunoassays would significantly diminish the labor necessary to obtain M antigens which are pure enough to be useful in the immunoassays, and would result in high yields of the M antigen.

A need presently exists for biological reagents which can be produced and purified quickly and safely, and in large quantities, and which can be used in diagnostic assays to rapidly, easily and accurately detect a previous or current infection by *H. capsulatum*, and to diagnose histoplasmosis. A need also presently

exists for a method of rapidly, easily and accurately detecting a previous or current infection by *H. capsulatum*, and to diagnose histoplasmosis. Such biological reagents and methods would allow a clinician 5 to improve the speed and accuracy of processing large numbers of clinical samples. Such reagents and methods would also aid the clinician in patient management, eliminate unnecessary tests, improve the speed, ease and accuracy of diagnosis and prognosis, help control 10 histoplasmosis infection and reduce the use of unnecessary medications.

Accordingly, the present invention provides the DNA nucleotide sequence of the M antigen gene of *H. capsulatum*, and of related nucleotide sequences, which 15 can be used to safely and rapidly produce, by recombinant DNA techniques, large quantities of the M antigen of *H. capsulatum* when inserted into a vector and placed into a suitable host for protein expression. The recombinantly-produced M antigens may be quickly and safely produced in 20 large quantities in a pure, undegraded form. The present invention also provides the RNA nucleotide sequence which encodes the M antigen of *H. capsulatum*, and related nucleotide sequences. Nucleic acids, and fragments thereof, within the invention can also be used as nucleic 25 acid probes in hybridization assays, or as primers in polymerase chain reaction assays, to detect *H. capsulatum* in clinical samples.

The present invention also provides the deduced amino acid sequence of the *H. capsulatum* M antigen. 30 Isolated and recombinant M antigens encoded by nucleic acids within the present invention can be used as biological reagents in a wide variety of tests for histoplasmosis, such as skin tests, and immunoassays to detect a previous or current *H. capsulatum* infection in a 35 tissue or fluid sample obtained from a human being or animal suspected of having, or having had,

histoplasmosis. For example, these antigens can be used as skin test antigens to ascertain the cell-mediated immune status of persons who have been exposed to *H. capsulatum*. The nucleic acids and antigens of the invention can also be used in a vaccine for the prevention or treatment of histoplasmosis.

The present invention also provides antibodies generated against the above antigens, which can be used in a wide variety of immunoassays to detect a current infection by *H. capsulatum*.

The present invention further provides methods for the detection of histoplasmosis, and related kits, using nucleic acids, antigens or antibodies within the invention.

The nucleic acids, vectors, hosts, isolated and recombinantly-produced antigens, antibodies, methods of detection and kits of the present invention permit the safe, direct, rapid, efficient, and accurate detection of a previous or current infection by *H. capsulatum* in a patient, and a positive diagnosis of histoplasmosis.

This patent application is believed to be the first report of the nucleotide sequence of the *H. capsulatum* M antigen gene, the nucleotide sequence which encodes the *H. capsulatum* M antigen, and of the amino acid sequence of the *H. capsulatum* M antigen.

DESCRIPTION OF THE RELATED ART

Zancopé-Oliveira et al., "Immunochemical Analysis of the H and M Glycoproteins from Histoplasma Capsulatum," *Clin. Diagn. Lab. Immunol.* Vol. 1, No. 5, 563-568 (1994), describes the use of different physicochemical methods to characterize the M and H antigens obtained from histoplasmin.

Zancopé-Oliveira et al., "Evaluation of Cation Exchange Chromatography for the Isolation of M Glycoprotein from Histoplasmin," *Journal of Medical and Veterinary Mycology* 31, 29-41 (1993), describes the development of chromatography procedures to isolate the M antigen from histoplasmin, and the monitoring of the physical, chemical and serological properties of the protein.

Zancopé-Oliveira et al., "Effects of Histoplasmin M. Antigen Chemical and Enzymatic Deglycosylation on Cross-Reactivity in the Enzyme-Linked Immunoelectrotransfer Blot Method," *Clinical and Diagnostic Laboratory Immunology* 1, No. 4, 390-393 (1994), describes an evaluation of the enzyme-linked immunoelectrotransfer blot (EITB) method as a suitable method for detecting antibodies present in sera from patients with histoplasmosis against M antigen, and the effect of chemical and enzymatic deglycosylation of M antigen as a means of increasing diagnostic specificity. The assay described in this article was stated to demonstrate 100% sensitivity with histoplasmosis serum samples, all of which were stated to react with the *H. capsulatum* M antigen.

Green et al. "Preparation of h and m Antigens of *Histoplasma capsulatum* Free of Heterologous Antigens," supra., describe the use of a salt gradient elution of crude histoplasmin on CM-sepharose CL6B at pH 3.0 in a one-step procedure to isolate the H, M and non-M antigens of *H. capsulatum*, and free them of any C antigen common to other pathogenic fungi to produce highly-purified antigens for use in immunoassays. This reference provides (Table 4 on Page 213) the gross amino acid composition (mole percent of sixteen amino acids) of the *H. capsulatum* M antigen, but not the amino acid sequence thereof.

Keath, "Molecular Cloning and Sequence Analysis of yps-3, a Yeast-Phase-Specific Gene in the Dimorphic

Fungal Pathogen *Histoplasma capsulatum*," *Microbiology* 140, 759-767 (1994), describes the cloning of the *H. capsulatum* yeast-phase-specific (*yps-3*) gene to clarify the mechanisms underlying pathogenesis and morphogenesis in the fungus *H. capsulatum*. The nucleotide sequence of the *yps-3* gene, and the predicted amino acid sequence of its product, are provided.

Deepe et al., "Immunobiological Activity of Recombinant H Antigen From *Histoplasma capsulatum*," *Infection and Immunity*, Vol. 63, No. 8, 3151-3157 (1995), describe the isolation and sequencing of the H antigen gene of *H. capsulatum*, and the recombinant production of the *H. capsulatum* H antigen in the bacterial expression vector pET 19b.

U.S. Patent No. 5,352,579 describes nucleic acid hybridization assay probes which are stated to be specific for *H. capsulatum* and no other fungi, and which have the nucleotide sequence 5' CGAAGTCGAGGGCTTCAGCATG3', or the nucleotide sequence complementary thereto. A probe having the above nucleotide sequence is stated to hybridize to the 18S rRNA of *H. capsulatum* corresponding to bases 172-193 of *Saccharomyces cerevisiae*. This patent also describes the use of helper probes having the sequence 5' TATTAGCTCTAGAATTACCACGGGTATCCAAGTAGTAAGG3', or the sequence 5' CCCCGAAGGGCATTGGTTTTATCTAATAAACACCCCC3'.

None of the above documents teaches or suggests the DNA nucleotide sequence of the *H. capsulatum* M antigen gene, the RNA nucleotide sequence which encodes the *H. capsulatum* M antigen, the amino acid sequence of the *H. capsulatum* M antigen, or the production of the *H. capsulatum* M antigen using recombinant DNA techniques.

SUMMARY OF THE INVENTION

The present invention provides the nucleotide sequence of the M antigen gene (DNA) of the *Histoplasma*

capsulatum species of fungus, which is set forth in the Sequence Listing as SEQ ID NO:1.

The present invention also provides a nucleic acid specific to *Histoplasma capsulatum* comprising a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, a fragment of a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, a fragment of a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, a fragment of a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, or a fragment of a nucleic acid having a nucleotide sequence which is substantially the same as a nucleic acid which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1. The isolated nucleic acid of this invention does not contain the nucleotide sequence

5' CGAAGTCGAGGCTTCAGCATG3', the nucleotide sequence

complementary thereto, the nucleotide sequence

5' TATTAGCTCTAGAATTACCACGGGTATCCAAGTAGTAAGG3', the nucleotide sequence complementary thereto, the nucleotide sequence

5' CCCCGAAGGGCATTGGTTTATCTAATAAACACCCCC3', or the

nucleotide sequence complementary thereto. Further, the isolated nucleic acid is not a nucleic acid consisting

essentially of between 10 and 100 nucleotides which is able to form a hybrid at 60°C with a nucleotide polymer having a nucleotide base sequence of

5 CGAAGTCGAGGCTTCAGCATG3 , 5 CATGCTGAAAGCCTCGACTTCG3 ,

5 CAUGCUGAAAGCCUCGACUUCG3 or 5 CGAAGUCGAGGCUUUCAGCAUG3 .

The present invention further provides the amino acid sequence of the isolated or recombinantly-produced M antigen of the *Histoplasma capsulatum* species of fungus, which is set forth in the Sequence Listing as SEQ ID

10 NO:2. The antigen is encoded by a nucleic acid (RNA) having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

The present invention also provides an isolated or recombinantly-produced antigen specific to *Histoplasma capsulatum* comprising a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary

to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, a fragment of a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set

forth in the Sequence Listing as SEQ ID NO:1, a

polypeptide encoded by a nucleic acid which is

substantially the same as a nucleic acid having a

25 nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, or a fragment of a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic

acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, or a

30 nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

The invention further provides monoclonal or polyclonal antibodies generated against one of the isolated or recombinantly-produced antigens described above.

The present invention also provides a vector comprising a nucleic acid specific to *Histoplasma capsulatum*, wherein the nucleic acid has a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, has a nucleotide sequence which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, is a fragment of a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, or 5 is a fragment of a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, and 10 wherein the vector is suitable for expressing the nucleic acid.

15 The present invention still further provides a host for expressing an antigen which is specific to *Histoplasma capsulatum* comprising a vector containing a nucleic acid, wherein the vector is suitable for expressing the nucleic acid, and wherein the nucleic acid 20 is as described above for the vector of the present invention.

The present invention also provides a vaccine for the treatment or prevention of histoplasmosis comprising:
(a) a nucleic acid, or an isolated or recombinantly-produced antigen, which is specific to *Histoplasma capsulatum*; and (b) a pharmaceutically-acceptable carrier for the nucleic acid or antigen, wherein the nucleic acid 25 in a nucleic acid as described above, and wherein the antigen is an antigen as described above.

30 The present invention further provides a method for detecting a previous or current *Histoplasma capsulatum* infection in a subject, comprising: (a) contacting a fluid or tissue sample from the subject which contains antibodies with an isolated or recombinantly-produced antigen which is specific to *Histoplasma capsulatum*; and 35 (b) detecting the presence of binding between the

antibodies and the antigen, the presence of binding indicating the presence of a previous or current *Histoplasma capsulatum* infection in a subject, wherein the antigen is one of the antigens described above.

5 The present invention further provides a method for detecting a past exposure to the fungus *Histoplasma capsulatum* comprising: (a) injecting intradermally in the skin of a subject a liquid containing an isolated or recombinantly-produced antigen which is specific to
10 *Histoplasma capsulatum*; and (b) observing the skin of the subject at the injection site at one or more predetermined times after injection for the presence of swelling of the skin, the presence of swelling of the skin indicating a past exposure by the subject to the fungus *Histoplasma capsulatum*, wherein the antigen is one of the antigens described above.

15 The present invention still further provides a kit for detecting a previous or current *Histoplasma capsulatum* infection in a sample comprising: (a) a nucleic acid, an isolated or recombinantly-produced antigen, or an antibody described above; and (b) instructions describing the use of the nucleic acid, antigen or antibody in the detection of a previous or current *Histoplasma capsulatum* infection.

20 The present invention also provides a method for detecting a current *H. capsulatum* infection in a subject suspected of having an *H. capsulatum* infection comprising: (a) contacting a fluid or tissue sample from the subject which contains antigens with antibodies
25 generated against an antigen which contains an epitope which is unique to *H. capsulatum*; and (b) detecting the presence of binding between the antigens and the antibodies, the presence of binding indicating the presence of a current *H. capsulatum* infection in the
30 subject, wherein the antigen is one of the antigens described above.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention may be understood more readily by reference to the following detailed description of the preferred embodiments of the invention, and to the

- 5 Example and Sequence Listing included therein.

Definitions

The phrases "specific to" and "unique to" the fungus *H. capsulatum* as used herein in relation to an antigen means that the antigen (an antigenic polypeptide or polypeptide fragment) contains at least one epitope which is not common to other related fungi or other microorganisms (i.e., it is unique to the fungus *H. capsulatum*), and binds with a higher affinity to antibodies generated against antigens of the fungus *H. capsulatum* than with antibodies generated against other related fungi or microorganisms. Thus, such an antigen can be distinguished from other antigens by such higher binding affinity. The phrases "specific to" and "unique to" the fungus *H. capsulatum* as used herein in relation to a nucleic acid or nucleic acid fragment means a nucleic acid or nucleic acid fragment which is not common to other related fungi or other microorganisms (i.e., it is only present in the fungus *H. capsulatum*).

The phrase "fully complementary" as used herein refers to a nucleic acid which is both the same length as, and exactly complementary in base pairing to, a given nucleic acid.

The phrase "fluid or tissue sample" as used herein means any sample of fluid, or of solubilized or nonsolubilized tissue, obtained from a subject, or solubilized or nonsolubilized cultured cells, which

contains components, such as nucleic acids, antibodies or antigens, or fragments thereof, which may be employed in one of the tests described herein to detect a previous or current infection by, or exposure to, the fungus *H.*

5 *capsulatum*, or to make a positive diagnosis of histoplasmosis. Such fluid or tissue samples include blood, serum, plasma, sputum, urine, mucus, saliva, gastric juice, lymph, feces, or other bodily fluids, and tissues from the lungs, spleen, liver, skin or other
10 organs. The tissue or fluid samples can also be supernatant from incubated tissue samples or cultured cells.

15 The term "fragment" as used herein in relation to a polypeptide means a subsequence of the polypeptide which is of a sufficient size and conformation to remain immunogenic (i.e., to have at least one epitope) and/or to produce swelling of the skin of a subject in a skin test for histoplasmosis. The term "fragment" as used herein in relation to a nucleic acid means a subsequence
20 of the nucleic acid which is of a sufficient size and confirmation to properly function as a hybridization probe, as a primer in a polymerase chain reaction, to code for a polypeptide or polypeptide fragment, or in another manner characteristic of nucleic acids.

25 The term "hybridization" as used herein refers to the formation of a duplex structure by two single-stranded nucleic acids due to fully (100%) or less than fully (less than 100%) complementary base pairing. Hybridization can occur between fully complementary
30 nucleic acid strands, or between less than fully complementary nucleic acid strands which contain regions of mismatch due to one or more nucleotide substitutions, deletions or additions.

The terms "immunogenic" and "antigenic" as used herein mean that a polypeptide, or a fragment thereof, elicits a protective immune response, for example, the production of antibodies against the polypeptide, or 5 fragment thereof, in a subject to which it is administered. The polypeptide or polypeptide fragment will have at least one epitope present therein.

The term "isolated" means that the nucleic acids, nucleic acid fragments, polypeptides, polypeptide 10 fragments or antibodies are of sufficient purity so that they may be employed, and will function properly, in a clinical, diagnostic, experimental or other procedure, such as an immunoassay, a hybridization assay, an amplification reaction, or a skin test for 15 histoplasmosis. Many procedures are known by those of ordinary skill in the art for purifying nucleic acids, nucleic acid fragments, polypeptides, polypeptide fragments and antibodies from other proteins, contaminants, and materials with which they may normally 20 be associated prior to their use in various procedures. For example, the M antigen of *H. capsulatum* obtained from histoplasmin may be purified by standard chromatography procedures, such as cation-exchange chromatography or anion-exchange chromatography, to remove other antigens 25 (c and h antigens, etc.) and proteins, and other components, of histoplasmin therefrom. Recombinantly-produced *H. capsulatum* M antigen may be purified by bound nickel-ion exchange chromatography, or by a combination 30 of Fast Protein Liquid Chromatography (FPLC) using size exclusion chromatography and anion and/or cation exchange chromatography.

Abbreviations for "nucleotides" used herein follow the nomenclature described by the Nomenclature Committee for the International Union of Biochemistry,

"Nomenclature for Incompletely Specified Bases in Nucleic Acid Sequences," *Eur. J. Biochem.* 150:1-5 (1985), in which "A" represents adenine residues, "C" represents cytosine residues, "T" represents thymine residues, "G" 5 represents guanine residues, "I" represents deoxyinosine residues, "M" represents adenine or cytosine residues, "R" represents adenine or guanine residues and "Y" represents cytosine or thymine residues.

The terms "nucleic acid" and "oligonucleotide" 10 include polydeoxyribonucleotides (containing 2-deoxy-D-ribose), polyribonucleotides (containing D-ribose), and to any other type of polynucleotide which is an N glycoside of a purine or pyrimidine base, or modified purine or pyrimidine base. The terms "nucleic acid" and "oligonucleotide" are used interchangeably herein. These 15 terms refer only to the primary structure of the molecule. Thus, these terms include double- and single-stranded DNA, as well as double- and single-stranded RNA. Nucleic acids and oligonucleotides can be prepared by any 20 of several well-known methods. For example, they may be prepared by cloning and restriction of desired sequences, or by direct chemical synthesis by the phosphotriester methods described by Narang et al., *Meth. Enzymol.* 68:90-99 (1979) and Brown et al., *Meth. Enzymol.* 68:109-151 25 (1979); by the diethylphosphoramidite method described by Beaucage et al., *Tetrahedron Lett.* 22:1859-1862 (1981); or by the solid support method described in U.S. Patent No. 4,458,066. A review of nucleic acid syntheses 30 methods is provided in Goodchild, *Bioconjugate Chemistry* 1(3):165-187 (1990).

The term "polypeptide" as used herein means a sequence of four or more amino acids which is immunogenic and/or produces swelling of a subject's skin in a skin test for histoplasmosis, for example the M antigen 35 protein of *H. capsulatum*. The sequence of four or more

amino acids can be modified, for example, by chemical, enzymatic or other treatment which does not diminish the immunogenic activity of the polypeptide to any substantial extent.

5 The phrase "recombinant DNA techniques" as used herein means well-known techniques which permit the isolation and propagation of individual genes, such as the M antigen gene of *H. capsulatum*, and the efficient expression of their products, such as the M antigen of *H.* capsulatum, by plasmid or other expression vectors in various bacterial, yeast or mammalian host expression systems. General information concerning recombinant DNA techniques is present, for example, in Rodriguez et al., Recombinant DNA Techniques: An Introduction (The

15 Benjamin/Cummings Publishing Company, Inc., Menlo Park, California, 1983), in Maniatis et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1982), and in Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley-Interscience, (John Wiley and Sons, New York (1987; updated quarterly)). The phrase "recombinantly-produced" as used herein means produced by recombinant DNA techniques.

25 The term "substantially the same as" in relation to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, or to the nucleotide sequence fully complementary thereto, refers to a nucleic acid having a nucleotide sequence which is similar to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, or to the nucleotide sequence which is fully complementary thereto, and which retains the functions of such nucleic acid, but which differs from such nucleic acid by the substitution, deletion and/or addition of one or more nucleotides, and/or by the

incorporation of some other advantageous feature into the nucleic acid, such as a radio label or other label (biotin, etc.) for nucleic acid detection or immobilization. For example, the essential structure and function of a polypeptide 5 or polypeptide fragment encoded by a nucleic acid which is substantially the same as the above nucleic acids should be the same as the structure and function of a polypeptide or polypeptide fragment encoded by the above nucleic acids. Generally, these nucleic acids will have a nucleotide sequence 10 which has less than about 10% divergence from the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, or from the nucleotide sequence which is fully complementary thereto. Preferably, the nucleic acids will have about 90%, or more preferably about 95%, 15 or even more preferably about 99% homology with the nucleotide sequence set forth in the Sequence Listing

as SEQ ID NO:1, or with the nucleotide sequence fully complementary thereto.

Due to the degeneracy in the genetic code, a sequence of three nucleotides (a codon) codes for each of the twenty natural amino acids. However, because there are twenty amino acids and sixty-four possible codons, most amino acids are specified by more than one codon. Thus, the nucleotide sequence of the *H. capsulatum* M antigen gene may be varied from the nucleotide sequence 20 set forth in the Sequence Listing as SEQ ID NO:1, and the nucleotide sequence which encodes the *H. capsulatum* M antigen may be varied from the nucleotide sequence which is fully complementary to the nucleotide sequence set 25 forth in the Sequence Listing as SEQ ID NO:1. Thus, nucleic acids within the present invention are not limited to nucleic acids having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, or having a nucleotide sequence fully complementary thereto. 30

The nucleic acids of the present invention will have the ability of the nucleic acid whose nucleotide sequence is set forth in the Sequence Listing as SEQ ID NO:1, or

whose nucleotide sequence is fully complementary thereto, to encode the *H. capsulatum* antigen gene, or M antigen product of this gene, with the M antigen being specific to *H. capsulatum* and being antigenic (being able to stimulate the production of antibodies against the antigen). Alternatively, the nucleic acids of the present invention will have the ability to function as hybridization probes, or as primers in amplification reactions, for the detection of *H. capsulatum*.

Modifications at the 5'- end of a nucleic acid can include, for example, the addition of an isotope, such as ^{32}P , or a chemical, such as digoxigenin, for detection when using a commercial kit, such as the Boehringer-Mannheim Dig/Genius detection system. In addition, restriction enzyme sites and/or cloning sites can be

added to the 5'- end of a nucleic acid (from about 6 to more than about 12 nucleotides) for the direct cloning of the amplified product.

The phrases "target region" and "target nucleic acid" refer to a region of a nucleic acid which is to be amplified, detected, or otherwise analyzed. The sequence to which a primer hybridizes is referred to as a "target sequence."

Nucleic Acids

In one aspect, the present invention provides nucleic acids which are specific to the fungus *H. capsulatum*.

Examples of the nucleic acids of the present invention include a DNA having the nucleotide sequences set forth in the Sequence Listing as SEQ ID NO:1, an RNA having a nucleotide sequence which is fully complementary

to the DNA nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, and fragments of the foregoing nucleic acids.

Modified Nucleic Acids

5 Nucleic acids within the present invention also include nucleic acids which are substantially the same as the nucleic acids having the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, or the nucleotide sequence which is fully complementary to the
10 nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

15 Modifications to a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, or to a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, such as one or more nucleotide substitutions, additions, and/or deletions, or the addition of some beneficial component to the nucleic acid, such as a radiolabel or non-
20 radiolabel for nucleic acid detection or immobilization, can be made so long as the nucleic acids do not lose their ability to function in one of the manners described herein. Such modified nucleic acids are within the scope of the present invention if they have the ability to
25 function to encode the *H. capsulatum* M antigen gene, to encode an antigenic polypeptide which is specific to *H. capsulatum*, to function as a nucleic acid probe in a hybridization assay for the detection of *H. capsulatum*, to function as a primer in a polymerase chain reaction
30 used to detect *H. capsulatum*, or to function in some other manner which is characteristic of nucleic acids.

35 Computer programs are readily available to the skilled artisan which can be used to compare modified nucleotide sequences to previously published nucleotide sequences of *H. capsulatum* to select appropriate sequences for use. A computerized comparison of modified

sequences with known sequences catalogued in GENBANK, a computerized database, may be made using the commercially-available computer programs DNASIS (Hitachi Engineering, Inc.), Word Search or FASTA of the Genetics Computer Group (Madison, WI), which search the catalogued nucleotide sequences for similarities to the nucleic acid in question.

Nucleic Acid-Based Assay Techniques

The nucleic acids of the present invention can be used to detect a current *H. capsulatum* infection in a sample by any of a number of well-known nucleic acid-based detection techniques, such as hybridization techniques, polymerase chain reaction (PCR), reverse transcription polymerase chain reaction (RT-PCR), ligase chain reaction (LCR), nucleic acid sequencing techniques, electrophoretic and non-electrophoretic identification of nucleic acids, and the like. Alternatively, these nucleic acids can also be used in vectors to safely produce large quantities of the *H. capsulatum* M antigen in suitable host cells for use in the immunodiagnostic techniques and skin tests for histoplasmosis described herein. Thus, the nucleic acids of the present invention, which can vary in length, can be used as probes in nucleic hybridization assays for the detection of *H. capsulatum*, or as primers in polymerase chain reactions for the detection of *H. capsulatum*. It is also contemplated that the nucleic acids of the present invention can be labeled or tagged for use in radioactive, chemiluminescence, fluorescent, or other detection systems.

H. capsulatum infection in a tissue or fluid sample suspected of containing *H. capsulatum* infection may be detected by detecting nucleic acids of *H. capsulatum*. Based upon the nucleotide sequence set forth in SEQ ID NO:1, one can design reagents by known methods to detect the presence of *H. capsulatum* in a sample. For example, DNA or RNA obtained from a sample suspected of containing

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H. capsulatum can be sequenced by known methods, and the sequence compared to the nucleotide sequence set forth in SEQ ID NO:1. If the sequence of DNA or RNA obtained from the sample has greater than about 10% divergence from the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, or from a nucleotide sequence complementary thereto, then the sample does not contain *H. capsulatum*. Otherwise (if there is about 90% or more sequence similarity between DNA or RNA obtained from the sample and the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, or the nucleotide sequence complementary thereto), a positive diagnosis of current infection in the sample by the fungus *H. capsulatum* can be made. The above-described computer programs may be used to make the nucleotide sequence comparisons.

Amplification reactions can also be used for detecting *H. capsulatum* infection in a sample. DNA obtained from the sample can be amplified using nucleic acid primers specific to *H. capsulatum*, and detecting the presence of a nucleic acid which is unique to *H. capsulatum*. The presence of a nucleic acid which is unique to *H. capsulatum* indicates the presence of *H. capsulatum* in the sample. The detection of a nucleic acid which is unique to *H. capsulatum* can be by the detection of amplification product when *H. capsulatum*-specific primers are used. The detection of a nucleic acid unique to *H. capsulatum* can be performed by direct hybridization utilizing a *H. capsulatum*-specific oligonucleotide probe, or by a restriction fragment length polymorphism. The primers (and probes) can, for example, be derived from the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, or the sequence complementary thereto. Particularly useful regions of the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1 for such purpose are (1) the DNA at the amino terminus encoding amino acids

1-24 of the M antigen, or the sequence complementary thereto; and (2) the DNA at the carboxy terminus encoding amino acids 601-707 of the M antigen, or the sequence complementary thereto. Standard criteria for the
5 selection of sequences for primer development are applicable. The crucial requirement is that the primers be such that an amplification protocol using them can distinguish *H. capsulatum* nucleic acids from the nucleic acids of other fungi, and other microorganisms. While
10 non-specific amplification may also occur, the skilled artisan can distinguish non-specific amplification from the amplification of nucleic acids of *H. capsulatum*, for example, by following amplification with the use of a specific probe derived from the nucleotide sequence set
15 forth in the Sequence Listing as SEQ ID NO:1, or to a sequence fully complementary thereto.

For such uses, the nucleic acids are typically between about 10 and about 100 nucleotides in length, preferably between about 12 and about 30 nucleotides in length, and most preferably between about 15 and about 25 nucleotides in length. There is no standard length for optimal hybridization or polymerase chain reaction amplification. An optimal length for a particular primer application may be readily determined in the manner
20 described in H. Erlich, PCR Technology, Principles and Application for DNA Amplification, (1989). Several computer software programs are available to facilitate primer design, for example, Lowe, "Computer Program for Selection of Oligonucleotide Primers for Polymerase Chain
25 Reactions," *Nucl. Acids. Res.* 18:1757-1761 (1991) and RT-PCR, Methods and Applications Book 1, (Clontech Laboratories, Inc. (1991)).

In particular, an isolated nucleic acid that selectively hybridizes with (or selectively amplifies)
35 the nucleic acid set forth in SEQ ID NO:1, or the nucleic acid fully complementary thereto, under stringent

conditions, and comprises at least 10 nucleotides complementary to the sequence set forth in SEQ ID NO:1, or the nucleic acid fully complementary thereto, is provided. The hybridizing nucleic acid should have at 5 least about 97% (and preferably about 98% or 99%) complementarity with the segment of the nucleic acid of SEQ ID NO:1, or the nucleic acid fully complementary thereto, to which it hybridizes. As used herein to describe nucleic acids, the term "selectively hybridizes" 10 means that a nucleic acid hybridizes with a particular nucleotide sequence, and not with others, and excludes the occasional randomly hybridizing nucleic acids. The hybridizing nucleic acids can be used, for example, as probes or primers for detecting an isolate of *H.* 15 *capsulatum* that has the nucleic acid to which the primer or probe hybridizes. Thus, these nucleic acids can be the coding sequence for the *H. capsulatum* M antigen protein, or for fragments thereof, that can be utilized to produce an antigenic protein or protein fragment. 20 If used as primers, the invention provides compositions including at least two nucleic acids which hybridize with different regions of the target *H.* *capsulatum* sequence so as to amplify a desired region of the target *H. capsulatum* sequence. Depending on the 25 length of the probe or primer, the target region can range from about 97% complementary bases and full complementarity and still hybridize under stringent conditions. For example, for the purpose of diagnosing the presence of *H. capsulatum* infection, the degree of 30 complementarity between the hybridizing nucleic acid (probe or primer) and the sequence to which it hybridizes (e.g., *H. capsulatum* DNA from a sample) is at least enough to distinguish hybridization with a nucleic acid from related fungi. 35 In general, the nucleic acids of the present invention may be prepared and tested for the ability to selectively hybridize with a target nucleic acid in the

manner described herein, or by modifications thereof, using readily-available starting materials, reagents and equipment.

The polymerase chain reaction (for amplifying DNA) and the reverse transcription polymerase chain reaction (for amplifying cDNA generated from RNA) are rapid methods for increasing the copy number of, and sensitively detecting, specific nucleic acid sequences. These methods may be used for the rapid detection of *H. capsulatum* from clinical samples.

The nucleic acids present in a sample which are being amplified may be a single- or double-stranded DNA or RNA. If the starting material is RNA, reverse transcriptase is used to prepare a first strand cDNA prior to conventional polymerase chain reaction.

General information concerning polymerase chain reaction, and the amplification of specific sequences of nucleic acids, is present in U.S. Patent No. 4,683,195; U.S. Patent No. 4,683,202; U.S. Patent No. 4,965,188; U.S. Patent No. 5,578,467; U.S. Patent No. 5,545,522; U.S. Patent No. 5,624,833; Ausubel et al., Current Protocols in Molecular Biology, supra.; Rotbart, "Enzymatic RNA Amplification of the Enteroviruses," *J. Clin. Microbiol.* 28:438-442 (1990); Kawasaki, "Amplification of RNA," 21-27, in M. Innis et al., PCR Protocols (Academic Press, New York (1990)); and Rossolini et al., "Use of Deoxyinosine-Containing Primers vs. Degenerate Primers for Polymerase Chain Reaction Based on Ambiguous Sequence Information," *Mol. Cell Probes* 8:91-98 (1994). The amplification of cDNA generated from RNA using a reverse transcription/polymerase chain reaction is described in U.S. Patent No. 5,310,652 and U.S. Patent No. 5,322,770. Commercial vendors, such as Perkin Elmer (Norwalk, Connecticut), market polymerase chain reaction reagents and equipment and publish suitable protocols.

In each cycle of an amplification reaction, a double-stranded target nucleic acid sequence present in a sample is denatured and, due to the presence of a large molar excess of the primers, primers are annealed to each strand of the denatured target sequence. The primers, oriented with their 3' ends pointing towards each other, hybridize to opposite strands of the target sequence and, due to the action of DNA polymerase, prime enzymatic extension along the nucleic acid template in the presence of the four deoxyribonucleotide triphosphates. The two primers anneal to opposite ends of the target nucleic acid sequence, and in orientations such that the extension product of each primer is a complementary copy of the target nucleic acid sequence and, when separated from its complement, can hybridize to the other primer. The end product is then denatured again for another cycle. After this three-step cycle has been repeated between about 25 and 40 times, amplification of a nucleic acid segment by more than one million-fold can be achieved. Each cycle, if 100% efficient, would result in a doubling of the number of target sequences present, thereby leading to exponential increases in the concentration of desired nucleic acid sequences. Better amplification is generally obtained when both primers are approximately the same length.

Denaturation of nucleic acid strands usually takes place at about 94°C. The normal annealing (55 to 60°C) and extension (65 to 72°C) temperatures generally used for *in vitro* amplification by polymerase chain reaction may be used. Examples of suitable reaction times are from about 30 seconds to about 1 minute denaturing; from about 30 seconds to about 1 minute of annealing; and from about 30 seconds to about 2 minutes of extension. One of ordinary skill in the art can, of course, easily determine optimum reaction times and conditions using conventional techniques.

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Suitable assay formats for detecting amplification products or hybrids formed between probes and target nucleic acid sequences in a sample are described, for example, in Ausubel et al., Current Protocols in Molecular Biology, supra., and in Sambrook et al., Molecular Cloning-A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1985). Examples of these assay formats include the dot-blot and reverse dot-blot assay formats. In a dot-blot format, amplified target DNA is immobilized on a solid support, such as a nylon membrane. The membrane-target complex is incubated with labeled probe under suitable hybridization conditions, unhybridized probe is removed by washing under suitable stringent conditions, and the membrane is monitored for the presence of bound probe. In a "reverse" dot-blot format, in which the amplified target DNA is labeled and the probes are immobilized on a solid support (e.g., nylon membrane). The target DNA is typically labeled during amplification by the incorporation of labeled primers therein. One or both of the primers can be labeled. The membrane-probe complex is incubated with the labeled amplified target DNA under suitable hybridization conditions, unhybridized target DNA is removed by washing under suitably stringent conditions, and the filter is then monitored for the presence of bound target DNA.

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"Stringent conditions" refers to the hybridization conditions used in a hybridization protocol, for example, DNA/DNA hybridization, or in the primer/template hybridization in a PCR reaction. In general, these conditions should be a combination of temperature and salt concentration for washing chosen so that the denaturation temperature is approximately 5-20°C below the calculated T_m (melting/denaturation temperature) of the hybrid under study. The temperature and salt conditions are readily determined empirically in

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preliminary experiments in which samples of reference DNA
are hybridized to the primer nucleic acid of interest,
and then amplified under conditions of different
stringencies. The stringency conditions are easily
5 tested, and the parameters altered will be apparent to
one skilled in the art. For example, MgCl₂ concentrations
used in the reaction buffer can be altered to increase
the specificity with which the primer binds to the
template, but the concentration range of this compound
10 used in hybridization reactions is narrow and, therefore,
the proper stringency level is easily determined. For
example, hybridizations with oligonucleotide probes 18
nucleotides in length can be done at 5-10°C below the
estimated T_m in 6X SSPE, then washed at the same
15 temperature in 2X SSPE. (See, e.g., Sambrook et al.,
Molecular Cloning: A Laboratory Manual, supra.) The T_m of
such an oligonucleotide can be estimated by allowing 2°C
for each A or T nucleotide, and 4°C for each G or C. An
18 nucleotide probe of 50% G+C would, therefore, have an
approximate T_m of 54°C. Likewise, the starting salt
20 concentration of an 18 nucleotide primer or probe would
be about 100-200mM. Thus, stringent conditions for such
an 18 nucleotide primer or probe would be a T_m of about
54°C, and a starting salt concentration of about 150 mM,
and modified accordingly by preliminary experiments. T_m
25 values can also be calculated for a variety of conditions
utilizing commercially available computer software (e.g.,
OLIGO®).

Conventional techniques of molecular biology and
30 nucleic acid chemistry which may be employed in the
preparative and testing processes of the present
invention are fully explained in the literature. See,
for example, Ausubel et al., Current Protocols in
Molecular Biology, supra. Sambrook et al., Molecular
35 Cloning-A Laboratory Manual, supra.; Watson et al.,
Molecular Biology of the Gene (Fourth Edition, The

Benjamin/Cummings Publishing Company, Inc. 1987);
Oligonucleotide Synthesis (M. J. Gait, ed., 1984); and
Nucleic Acid Hybridization (B. D. Hames and S. J.
Higgins. eds., 1984).

5 **Vectors and Hosts**

The present invention also provides a vector comprising a nucleic acid having the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, having a nucleotide sequence which is substantially the same as 10 the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, a nucleic acid complementary to, or capable of hybridizing with, either of the foregoing nucleic acids, or a fragment of any of the foregoing nucleic acids. The vectors of the invention can be 15 placed into a host (e.g., cell line or transgenic animal) that can express the polypeptides and polypeptide fragments of the present invention.

The *H. capsulatum* M antigen gene (and other nucleic acids within the invention) can be cloned into suitable expression vectors by linking the gene to a suitable promoter in a replicable vector, and expressed in various bacterial, yeast or mammalian host expression systems, as is described in the Example, to safely produce large quantities of the *H. capsulatum* M antigen by propagating 20 the vector in the host under conditions conducive to protein expression. Using conventional techniques, a DNA sequence containing the *H. capsulatum* M antigen gene can be cloned from *H. capsulatum* genomic DNA. The DNA can be converted to double-stranded DNA using cloning techniques 25 well known in the art, including PCR techniques. Linkers or tails may be placed on the ends of the double-stranded DNA to provide convenient restriction sites. After restriction digestion, the DNA may be introduced to any site in a vector, such as a plasmid vector, which has 30 been restricted with a restriction enzyme which generates 35

compatible ends. Following ligation, by means of standard techniques, the DNA can then be introduced into a suitable host system, where it can be expressed to produce the desired *H. capsulatum* M antigen protein.

5 If desired, the coding sequence for the *H. capsulatum* M antigen gene can be subjected to site-specific mutagenesis, in the manner discussed by Maniatis et al., Molecular Cloning: A Laboratory Manual, supra., to alter selected base pairs. Oligonucleotides
10 containing a mutation to be introduced to the cloned gene can be synthesized by well-known DNA synthetic techniques, preferably by phosphoramidite chemistry, and preferably as implemented on an automated synthesizer, such as the synthesizer commercialized by Applied Biosystems.
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There are numerous *E. coli* expression vectors known to those of ordinary skill in the art which are useful for the expression of the polypeptides and polypeptide fragments of the invention. Other microbial hosts suitable for such use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia* and various *Pseudomonas* species. In these prokaryotic hosts, one can also make expression vectors which contain expression control sequences
20 compatible with the host cell, such as an origin of replication. In addition, any number of a variety of well-known compatible promoters will be present, such as a lactose promoter system, a tryptophan (Trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically
25 control expression, optionally with an operator sequence, and have ribosome binding site sequences for initiating and completing transcription and translation. If necessary, an amino terminal methionine can be provided
30 by the insertion of a Met codon 5' in-frame with the polypeptide or polypeptide fragment. Also, the carboxyl-

terminal extension of the antigen can be removed using standard oligonucleotide mutagenesis procedures.

Additionally, yeast expression systems can be used for the recombinant production of the polypeptide or polypeptide fragment. There are several advantages to the use of yeast expression systems for this purpose. First, evidence exists that proteins produced in a yeast secretion system generally exhibit correct disulfide pairing. Second, post-translational glycosylation is generally efficiently carried out by yeast secretory systems. The *Saccharomyces cerevisiae* pre-pro-alpha-factor leader region (encoded by the *MFα-1* gene) is routinely used to direct protein secretion from yeast. (See, for example, Brake et al., "α-Factor-Directed Synthesis and Secretion of Mature Foreign Proteins in *Saccharomyces cerevisiae*," *Proc. Nat. Acad. Sci.* 81:4642-4646 (1984)). The leader region of pre-pro-alpha-factor contains a signal peptide and a pro-segment which includes a recognition sequence for a yeast protease encoded by the *KEX2* gene. This enzyme cleaves the precursor protein on the carboxyl side of a Lys-Arg dipeptide cleavage-signal sequence. The antigen coding sequence can be fused in-frame to the pre-pro-alpha-factor leader region. This construct is then put under the control of a strong transcription promoter, such as the alcohol dehydrogenase I promoter or a glycolytic promoter. The antigen coding sequence is followed by a translation termination codon, which is followed by transcription termination signals. Alternatively, the antigen coding sequences can be fused to a second protein coding sequence, such as Sj26 or β-galactosidase, used to facilitate purification of the fusion protein by affinity chromatography. The insertion of protease cleavage sites to separate the components of the fusion protein is applicable to constructs used for expression in yeast. Efficient post translational glycosylation and expression

of recombinant proteins can also be achieved in Baculovirus systems.

Mammalian cells permit the expression of proteins in an environment which favors important post-transitional modifications, such as folding and cysteine pairing, the addition of complex carbohydrate structures, and the secretion of active protein. Vectors useful for the expression of antigen in mammalian cells are characterized by insertion of the antigen coding sequence between a strong viral promoter and a polyadenylation signal. The vectors can contain genes conferring either gentamicin or methotrexate resistance for use as selectable markers. The antigen coding sequence can be introduced, for example, into a Chinese hamster ovary cell line using a methotrexate resistance-encoding vector. The presence of the vector DNA in transformed cells can be confirmed by Northern blot analysis, and the production of an opposite strand RNA corresponding to the antigen coding sequence can be confirmed by Southern blot analysis. A number of other suitable host cell lines capable of secreting intact human proteins have been developed, and include the CHO cell lines, HeLa cells, myeloma cell lines, Jurkat cells, and the like. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer, and necessary information processing sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from immunoglobulin genes, SV40, Adenovirus and Bovine Papilloma Virus. The vectors containing the nucleic acid segments of interest can be transferred into the host cells by well-known methods, which vary depending upon the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium

phosphate treatment or electroporation may be used for other cellular hosts.

Alternative vectors for the expression of antigen in mammalian cells, such as those which are similar to the vectors developed for the expression of human gamma-interferon, tissue plasminogen activator, clotting Factor VIII, hepatitis B virus surface antigen, protease Naxinl, and eosinophil major basic protein, can also be employed. Further, the vector can include CMV promoter sequences and a polyadenylation signal available for expression of inserted nucleic acid in mammalian cells, such as COS7.

The nucleic acid sequences can be expressed in hosts after the sequences have been operably linked, i.e., positioned, to ensure the functioning of an expression control sequence. These expression vectors are typically replicable in the host organisms either as episomes, or as an integral part of the host chromosomal DNA. Commonly, expression vectors can contain selection markers, e.g., tetracycline resistance or hygromycin resistance, to permit detection and/or selection of those cells transformed with the desired nucleic acid sequences. (See, for example, U.S. Patent 4,704,362).

Nucleic acids encoding a variant polypeptide may include sequences which facilitate transcription (expression sequences) and translation of the coding sequences, such that the encoded polypeptide product is produced. Construction of such nucleic acids is well known in the art. For example, such nucleic acids can include a promoter, a transcription termination site (polyadenylation site in eukaryotic expression hosts), a ribosome binding site and, optionally, an enhancer for use in eukaryotic expression hosts and sequences necessary for replication of a vector.

Antigens and Methods

The antigens of the present invention, and monoclonal or polyclonal antibodies raised or generated against these antigens, are useful as diagnostic reagents for detecting the presence of the fungus *H. capsulatum* in a sample, the presence of a previous or current infection by *H. capsulatum*, and for diagnosing histoplasmosis.

Numerous assay techniques based upon immunological reactions between antigens and antibodies may be performed with the antigens and antibodies of the invention to detect the presence of *H. capsulatum* in a sample, the presence of a previous or current infection by *H. capsulatum*, and for making a positive diagnosis of histoplasmosis, including the well-known enzyme-linked immunosorbent assays (ELISA), immunofluorescence assays (IFA), radioimmuno assays, immunoelectrophoresis, immunoblotting and the like.

Using any of the known assay techniques which are based upon immunological reactions, a previous or current *H. capsulatum* infection in a subject may be detected by the steps comprising: (a) contacting a fluid or tissue sample from the subject which contains antibodies with an isolated or recombinantly-produced antigen of the present invention; and (b) detecting the presence of binding between the antibodies and the antigen, the presence of binding indicating the presence of a previous or current *H. capsulatum* infection in the subject.

In these immunodiagnostic techniques, the antigen employed can be any of the isolated or recombinantly-produced polypeptides or polypeptide fragments described hereinabove. Because large quantities of polypeptides and polypeptide fragments can be safely produced by recombinant DNA techniques using nucleic acids described herein, and purified, it is preferable to use recombinantly-produced polypeptides and polypeptide

fragments in the immunodiagnostic techniques of the invention.

The nucleotide sequence which is complementary to the DNA nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1 encodes the *H. capsulatum* M antigen. Thus, the antigen employed in the immunoassay techniques described herein can be this protein, an antigenic polypeptide fragment of this protein, or any other antigenic polypeptide or polypeptide fragment encoded by nucleic acid which has a nucleotide sequence which is complementary to SEQ ID NO:1, or to a nucleic acid which has a nucleotide sequence which is substantially the same as the nucleotide sequence which is complementary to SEQ ID NO:1. It is already well established that the *H. capsulatum* M antigen is antigenic, and is specific for *H. capsulatum*. Fragments of the *H. capsulatum* M antigen may also possess one or more epitopes of the M antigen protein which are unique to *H. capsulatum*. These epitopes, and the polypeptides and polypeptide fragments containing them, can be readily determined by the well-known techniques of epitope mapping and conformational dependency analysis.

Monoclonal antibodies directed against the M antigen may be utilized, as described in Zancopé-Oliveira et al., "Evaluation of Cation Exchange Chromatography for Purifying the M-glycoprotein Antigen from Histoplasmin," *J Med Vet Mycol* 31, 29-41 (1993), and Zancopé-Oliveira et al., "Immunochemical Analysis of Glycosidic Epitopes in the H and M Antigens from *Histoplasma capsulatum*," *Clinical and Diagnostic Laboratory Immunology*, 1: 563-568 (1994). The monoclonal antibodies can be applied in the enzyme-linked immunoelectrotransfer blot (western blot) method. In addition, partial digestion with proteinases can be utilized to fragment recombinant M antigen. The fragments can be purified by Fast Protein Liquid Chromatography (FPLC), and used in an intermediate gel to

inhibit the immune precipitation of M antigen by specific antiserum in 2 dimensional crossed rocket immunoelectrophoresis. Further, a phage display library with restriction endonuclease digested M antigen gene can be developed. The phages expressing peptides can be tested by replica plating for immunoreactivity by indirect enzyme immunoassay. By testing homologous antisera and monoclonal antibodies, and those obtained from heterologous fungi, one can determine which peptide fragments contain epitopes specific for *Histoplasma capsulatum*.

Polypeptides which may be employed in the immunodiagnostic assays and skin tests of the present invention are those encoded by the plus strands of the nucleic acids of the invention. Antigenic fragments of the polypeptides can be synthesized directly, or obtained by chemical or mechanical disruption of the fungus, or of the larger polypeptides. The antigenic polypeptides and polypeptide fragments of the present invention can also be recombinant proteins, polypeptides or fragments thereof, obtained by cloning nucleic acids encoding the proteins, polypeptides or fragments in an expression system capable of producing the antigenic proteins, polypeptides, or fragments thereof.

Using the deduced amino acid sequence of the *H. capsulatum* M antigen set forth in the Sequence Listing as SEQ ID NO:2, it is also possible to synthesize, using standard peptide synthesis techniques, polypeptide fragments chosen to be homologous to immunoreactive regions of the larger antigen, and to modify these fragments by inclusion, deletion or modification of particular amino acids residues in the sequences. The amino acid sequences of the antigens of the invention can contain an immunoreactive region attached to sequences designed to provide for some additional property, such as solubility. These amino acid sequences can also include

amino acid substitutions to provide for some additional property, such as to remove or add amino acids capable of disulfide bonding, to increase antigenicity and/or bio-longevity, or to alter enzymatic activity. Thus,

5 synthesis and purification of an extremely large number of polypeptides and polypeptide fragments derived from the *H. capsulatum* M antigen is possible. However, these polypeptides and polypeptide fragments need to have a bioactive property, such as antigenicity.

10 The isolated polypeptides and polypeptide fragments obtained or produced can be tested to determine their antigenicity (immunoreactivity), immunogenicity and specificity by the well-known methods discussed hereinabove. One example of an immunologic technique
15 that may be used for the detection of current or previous infection by *H. capsulatum* utilizes monoclonal antibodies (MAbs) for detection of antibodies that specifically bind *H. capsulatum* M antigen. Briefly, sera or other body fluid from the subject is reacted with *H. capsulatum* M antigen bound to a substrate (e.g., an ELISA 96-well plate). After excess sera is thoroughly washed away, a labeled (e.g., enzyme-linked, fluorescent, radioactive, or the like) monoclonal antibody is then reacted with the previously reacted antigen-serum antibody complex. The
20 amount of inhibition of monoclonal antibody binding is measured relative to a control (no patient serum antibody).

25 The isolated or recombinantly-produced antigens of the invention can also be used as skin test antigens in skin tests for histoplasmosis. These skin tests are performed in a manner known by those of skill in the art for this disease, and for other pulmonary diseases, such as tuberculosis. Generally, a small quantity (generally about 0.1 ml) of liquid, such as physiological saline, containing an antigen of the invention, such as the *H. capsulatum* M antigen, is injected intradermally beneath

the skin (on the forearm or other convenient location) of
a patient, and the site of injection is observed at
predetermined times, such as 24 and 48 hours post
injection, for the presence of swelling of the skin. If
5 no swelling of the skin at the injection site is
observed, this indicates that the patient tested was not
exposed to *H. capsulatum*. If swelling of the skin at the
injection site is observed, this indicates that the
patient tested has been exposed to *H. capsulatum*. Skin
10 tests are usually observed, and the area of induration
measured, at 24 hours, 48 hours and 72 hours after
intradermal injection in the volar surface of the
forearm. For general information concerning these skin
tests, see Klimas, "Delayed Hypersensitivity Skin
15 Testing," pp. 276-280, in Rose et al., Manual of Clinical
Laboratory Immunology (5th ed., eds. American Society for
Microbiology, Washington, 1996). For general information
concerning the use of *H. capsulatum* glycoproteins in a
skin test for the diagnosis of histoplasmosis, see
20 Sprouse, "Determination of Molecular Weight, Isoelectric
Point, and Glycoprotein Moiety for the Principal Skin
Test-Reactive Component of Histoplasmin," *Infection and*
Immunity 15, 263-271 (1977).

Prior to using the isolated or recombinantly-
25 produced antigens in any immunodiagnostic assays or skin
tests, it is preferable that the antigens be partially or
fully deglycosylated by, for example, mild periodate
oxidation with about 0.025 M sodium meta-periodate at
about 4°C for about 4-8 hours in the dark, followed by
30 reduction with sodium borohydride, and then an equimolar
amount of glycerol.

Antibodies

An isolated antibody which binds with antigens of
the present invention is also provided. The antibodies
35 can be polyclonal or monoclonal, and should specifically

bind an epitope of an antigen which is specific to *H. capsulatum*. The term "bind" means the well-understood antigen-antibody interactions, or other nonrandom association with an antigen. "Specific binding" as used herein means an antibody that has a higher affinity for its target molecule (e.g., an antigen of the invention) than for non-target molecules (e.g., antigens of other closely-related fungi, or of other microorganisms).

Antibodies can be made by many well-known methods.

See, for example, Harlow and Lane, Antibodies; A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1988)). Briefly, an isolated or recombinantly-produced antigen can be injected into an animal in an amount, and in intervals, sufficient to elicit an immune response (i.e., the production of antibodies against the antigen). Antibodies can be obtained from the animal and purified directly by well-known methods. Alternatively, spleen cells can be obtained from the animal, and then fused with an immortal cell line and screened for monoclonal antibody secretion. The antibodies can be used to screen nucleic acid clone libraries for cells secreting the antigen. These positive clones can then be sequenced. The production of a murine monoclonal antibody (EC2-EC7) which is specific to the M antigen of *H. capsulatum* is described in Reiss et al., "Monoclonal Antibodies against the M-protein and Carbohydrate Antigens of Histoplasmin Characterized by the Enzyme-Linked Immunoassay Blot Method," *Infection and Immunity*, 53, 540-546 (1986).

Specific examples of isolated antibody within the invention which specifically bind to *H. capsulatum* antigens include antibodies which specifically bind with an isolated or recombinantly-produced polypeptide encoded by a nucleic acid which has a nucleotide sequence which is complementary to SEQ ID NO:1, or to antigenic fragments thereof.

Using any of the known assay techniques which are based upon immunological reactions, a current *H. capsulatum* infection in a subject suspected of having an *H. capsulatum* infection may be detected by the steps comprising: (a) contacting a fluid or tissue sample from the subject which contains antigens with antibodies generated against an antigen of the present invention; and (b) detecting the presence of binding between the antigens and the antibodies, the presence of binding indicating the presence of a current *H. capsulatum* infection in the subject.

General information concerning the reactions of antibodies to antigens of *H. capsulatum* is present in Kumar et al., "Cross-Reacting Human and Rabbit Antibodies to Antigens of *Histoplasma capsulatum*, *Candida Albicans* and *Saccharomyces Cerevisiae*," *Infect. Immun.* 48:806-812 (1985); Reiss et al., "Monoclonal Antibodies Against the M Protein and Carbohydrate Antigens of Histoplasmin Characterized by the Enzyme-Linked Immunoassay Transfer Blot Method," *supra.*; and Harris, "Characterization of Antigenic Determinants in Histoplasmin that Stimulate *Histoplasma Capsulatum*-Reactive T Cells in Vitro," *Infection and Immunity* 56, 2343-2349 (1988).

Kits

The present invention also provides a kit for detecting a previous or current *H. capsulatum* infection in a sample, or for diagnosing histoplasmosis. Preferably, the kit will contain one or more of the isolated nucleic acids, isolated or recombinantly-produced antigens, or isolated antibodies of the invention, and instructions describing the use of the nucleic acids, antigens or antibodies in the detection of a previous or current *H. capsulatum* infection, or in the diagnosis of histoplasmosis.

Vaccines

The isolated nucleic acids and isolated or recombinantly-produced antigens of the present invention may be used as the active component in an immunogenically-effective amount (an amount which is effective to stimulate the production of antibodies against the nucleic acids or antigens in the particular subject being vaccinated) in a vaccine for the prevention or treatment of histoplasmosis along with a pharmaceutically-acceptable carrier for the nucleic acids or antigens to provide protective resistance against *H. capsulatum*. Such a vaccine would be particularly useful for individuals who are at a high risk for contracting histoplasmosis, such as individuals who explore caves where birds and/or bats may be present, and individuals who deconstruct vacant buildings, which be inhabited by birds and/or bats.

Active immunization can be achieved through natural infection with an organism or virus, or artificially by vaccination. (See, for example, Kuby, Immunology (W.H. Freeman and Co., New York (1992))). It is also contemplated that immunization against disease caused by *H. capsulatum* can be achieved by a "naked" DNA vaccine approach. Briefly, DNA constructs containing promoter sequences upstream of *H. capsulatum* M antigen coding sequences can be injected into muscle tissue or administered via the mucosa and result in expression of antigens that induce a protective immune response.

An immunogenically-effective amount of the nucleic acids or antigens of the invention will generally range from about 100 nanograms to about 1 microgram of the nucleic acids, and from about 10 to about 100 micrograms of the antigens. Immunogenically-effective amounts of the vaccine, nucleic acid or antigen can be determined using standard procedures. Briefly, various concentrations of the nucleic acid or antigen are

prepared and administered to an animal, and then the immunological response (e.g., the production of antibodies or cell mediated immunity) of the animal to each concentration is determined. The amounts of nucleic acid or antigen administered depend on the subject, e.g. a human or an animal, the condition of the subject, the size of the subject, etc. Thereafter, the animal so inoculated with the nucleic acid or antigen can be exposed to *H. capsulatum* to test the potential vaccine effect (protective immunogenicity) of the specific nucleic acid or antigen. The specificity of the nucleic acid or antigen can be ascertained by testing sera, other fluids or lymphocytes from the inoculated animal for cross reactivity with other closely-related fungi, or other microorganisms.

The pharmaceutically-acceptable carrier which may be employed in the vaccines can comprise saline or other suitable carriers. See, for example, Arnon, R. (Ed.) Synthetic Vaccines (CRC Press, Inc., Boca Raton, Florida (1987)). By "pharmaceutically-acceptable" is meant a material that may be administered to a subject along with a selected nucleic acid or antigen without causing any undesirable biological effects, or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier will depend upon the method of administration and choice of adjuvant, if one is used. An adjuvant can also be a part of the carrier of the vaccine, in which case it can be selected by standard criteria based upon the nucleic acid or antigen used, the mode of administration and the subject. Methods of administration can be by oral or sublingual means, or by injection, depending on the particular vaccine used, and the subject to whom it is administered.

The vaccine can be used as a prophylactic or a therapeutic modality. Thus, the invention contemplates

methods of preventing or treating infection from *H. capsulatum*, and the associated diseases, by administering the vaccine to a subject.

The following Example describes and illustrates the methods for the cloning and sequencing of the *H. capsulatum* M antigen gene. This Example is intended to be merely illustrative of the present invention, and not limiting thereof in either scope of spirit. Those of skill in the art will readily understand that variations of the reagents used in, and the conditions and processes of, the procedures described in this Example can be used to clone and sequence nucleic acids which are specific to *H. capsulatum*.

All materials and equipment employed in the Example, and generally employed to make and use the nucleic acids, polypeptides, vaccines and kits of the present invention, are commercially-available. Sources for these materials and equipment are set forth in the Example, or are known by those of skill in the art.

EXAMPLE

Cloning and Sequencing of
the *H. capsulatum* Gene Encoding the M Antigen

In these experiments, the gene encoding the M antigen of *H. capsulatum* var. *capsulatum* (anamorph name, but also known by the teleomorph name *Ajellomyces capsulatus*) Centers for Disease Control and Prevention (CDC) strain 6623, which is deposited with the ATCC under Accession Number ATCC 26320, was cloned and sequenced.

Semi-purified M. protein was transferred to PVDF membranes. The eluted protein was sequenced directly, and cleaved with various proteinases, and the internal peptides were sequenced by microbore HPLC. Although the NH₂ terminus was blocked, several internal amino acid sequences were obtained. A homology search through a protein data base revealed significant similarity of these amino acid sequences to both eukaryotic and prokaryotic catalases. This degree of conservation facilitated peptide alignments. Degenerate oligonucleotides were constructed in the proper orientation for polymerase chain reactions (PCR). The amino acid sequence derived from the resulting amplicon confirmed that it encoded a region of the M antigen gene. This probe was used to screen an *H. capsulatum* genomic library, and a 4.0 kb fragment containing the entire M antigen gene was cloned and sequenced by the dideoxy chain termination method of Sanger et al., "DNA Sequencing with Chain-Terminating Inhibitors," Proc. Natl. Acad. Sci USA 74, 5463-5467 (1977). This gene was found to contain five introns, as determined by sequence analysis of cDNA obtained by reverse transcription polymerase chain reaction, and to be homologous with other members of the catalase family. The nucleotide sequence (DNA) for this M antigen gene, which contains

3862 nucleotides, is set forth in the Sequence Listing as SEQ ID NO:1. For the mature protein (not including a sixteen amino acid leader sequence), the open reading frame starts at base pair number 566 of the genomic
5 clone, and stops at base pair number 2812 thereof. Introns are present in the nucleotide sequence between six exons, which are present at base pair numbers 566-793, 852-1077, 1168-1583, 1706-1870, 1950-2124 and 2208-3121. The sequence of the clone containing the M
10 antigen gene has been deposited in GenBank under accession number AFO 26268.

Materials and Methods

Strains, plasmids and cultures conditions. Yeast-phase cells of *H. capsulatum* strain 6623 (ATCC 26320) were grown at 37°C in Pine's Liquid Medium for 48 hours to late log phase. *E.coli* strain q358 was used as the host for the bacteriophage 1 Gem11, and *E.coli* INV aF' (Invitrogen Co., Carlsbad, CA) was used as the recipient for the subcloning vector pBluescript SK (Stratagene, La Jolla, CA).

Purification of the M antigen. M antigen was purified by tandem cation exchange chromatography in columns of CM Sepharose CL-6B from histoplasmin, as described by Zancopé-Oliveira et al., "Evaluation of Cation Exchange Chromatography for the Isolation of M Glycoprotein from Histoplasmin," supra.

Amino acid sequence of M antigen. Samples of M antigen were electrophoresed on 10% SDS-PAGE, and transferred for 1 hour at 400 mA to polyvinylidene difluoride membranes (Immobilon-P, Milipore Corp., Bedford, Mass.) in 25 mM Tris, 192 mM glycine, and methanol (20% [vol/vol]). The membrane was washed several times with 1 mM DTT, stained with Ponceau S and destained

with 10% aldehyde-free acetic acid-1 mM DTT. Several washings with 1 mM DTT were made to remove the acetic acid. The band was identified by its molecular weight, and its identity was confirmed by immunoblotting. The
5 band corresponding to M antigen (200 pmol/protein band) bound to the membrane was excised, and submitted to Edman degradation without any prior modification. To obtain the internal sequences, the band was digested in situ with lysyl endopeptidase (Boehringer Mannheim,
10 Indianapolis, IN), and peptides were purified using microbore reverse-phase high-performance liquid chromatography (HPLC) on reverse phase C18 silica. All amino acid sequences were obtained using ABI sequencers (models 477A Protein Sequencer or Procise, Applied
15 Biosystems, Foster City, CA) which utilize pulse-liquid chemistry.

20 **DNA isolation.** Yeast cells grown in 50 ml of Pine's broth were harvested by filtration on 0.45 μm porosity membrane (Nalgene), washed 3 times with deionized H₂O and blotted to remove excess moisture. Cells were placed in a sterile mortar with approximately 1 g glass beads (0.5 mm), and liquid nitrogen, and were ground to a fine powder. The powder was resuspended in 20 ml of TE Buffer, pH 8.0 (10 mM Tris-1 mM EDTA), and DNA was
25 extracted with phenol, ethanol precipitated, and dried and redissolved in 0.05 M TE. The RNA was removed by the addition of RNase (10 $\mu\text{g}/\text{ml}$ final concentration) (Boehringer Mannheim) at 37°C for 1 hour, followed by proteinase K treatment (50 $\mu\text{g}/\text{ml}$) (Sigma Chemical Co.,
30 St. Louis, MO) for an additional 1 hour at 37°C. The DNA was subjected again to phenol extraction, and EtOH precipitation, and redissolved in TE.

35 **Generation of M DNA probe by PCR.** *H. capsulatum* genomic DNA was used to amplify a DNA fragment encoding an internal portion of the M protein by PCR. Degenerated

oligonucleotides primers ($1 \mu\text{M}$) were designed on the basis of two of six internal peptides (V22 and V18) derived from the amino acid sequence of the M antigen, which are set forth in Table 1, because the NH_2 terminus
5 appeared to be blocked:

Table 1

Amino Acid Sequences of NH_2 -Terminus and Lysyl Endopeptidase-Digested Fragments of the M Antigen

PCT/US94/03622 (1994-02-18)

	<u>Origin</u>	<u>Amino Acid Sequence</u>
10	<u>NH_2 terminus</u>	S D P T D Q F L (SEQ ID NO:3)
<u>Internal Sequences</u>		
	2642-m1947/19	D F I F R Q K I Q H F D H E R (SEQ ID NO:4)
	5070-m1941/20	T L Q G R A G L V (SEQ ID NO:5)
15	V22-m1947/20	A Q A L G G K N P D F H R Q D L (SEQ ID NO:6)
	V21-m1947/12	S G R Y P E (SEQ ID NO:7)
	V16-m1941/21	F D F D L L D P T K (SEQ ID NO:8)
	V18-m1941/23	I I P E E L V P F T P I G K (SEQ ID NO:9)

20 The sense primer M4F [5'-AA(AG)AA(CT)CC(AGC)GA(CT)TT(CT)-3', SEQ ID NO:10] was a 15-mer with 48-fold degeneracy,
and the antisense primer
M8R [5'-TT(AGCT)CC(AGT)AT(AGCT)GT(AG)AA-3', SEQ ID NO:11] was a 14-mer with 96-fold degeneracy. PCR was
25 carried out in a total volume of 100 μl containing 100 ng

of DNA as template, 100 M each of dNTP, 1 M of each oligonucleotide primer, and 10X PCR Buffer containing 500 mM KCl, 100 mM Tris-HCl, pH 8.3, 25 mM MgCl₂, and 2.5 U of Taq polymerase (Boehringer Mannheim). The
5 amplification conditions consisted of a denaturation at 95°C for 5 minutes followed for 35 cycles of the succeeding steps: denaturation at 95°C for 5 minutes, annealing at 50°C for 1 minute, and extension at 72°C for 1 minute. A final elongation was done at 72°C for 5
10 minutes. A 300-bp PCR product was subcloned into the pCRII vector using the TA cloning kit (Invitrogen, San Diego, Calif.), and using procedures recommended by the vendor, and sequenced using a dye-labeled terminator and automated sequencer (Applied Biosystems).

15 **Screening of an *H. capsulatum* genomic library.** The 300-bp amplicon was labeled with [-³²P]dCTP by High Prime DNA Labeling Mix (Boehringer Mannheim), purified in a DEAE column (NACS Prepac Convertible - BRL Life Technologies, Inc.), and used for screening the genomic library, derived from DNA partially digested with Sau3A1 and cloned into lGem11 via the *Xho* 1 half-site. An *E. coli* q358 strain bacterium infected with the genomic library, was replica plated onto nitrocellulose membranes. Plaques were lysed, and then heat fixed.
20 Filters were hybridized with ³²P-labeled probe. Twelve positive colonies were picked, and rescreened as large plaques. Two strongly positive plaques were purified and mapped by Southern analysis. These clones were digested with *BamH*1 and one fragment of 4.0 kb was obtained.
25

30 **Gene sequence analysis:** The 4.0 kb fragment was subcloned into pBluescript II KS, and sequenced by the strategy of ~~primer walking~~ using the dideoxy chain termination method. Oligonucleotides of 22-mer were synthesized on the basis of DNA sequence and applied to

initiate the sequence reaction. The clone was sequenced in both directions. To determine the sites of putative introns, 5 µg of RNA was reverse transcribed using oligo-dT to initiate the cDNA reaction. The first strand of
5 cDNA was amplified with a sense primer located at the start site of the mature protein: the sequence of this primer was 5'-CGGAATCCTCCGACCCCTACGGA-3' (SEQ ID NO:12). The antisense primer was 5'-ACCAAGCTTCTATCCAACGGGAACCGA-3' (SEQ ID NO:13). A 5'EcoRI site (underlined) was added to
10 the sense primer, and a HindIII site (underlined) was added to the antisense primer to facilitate cloning in pBluescript SK-. PCR was performed for 35 cycles of 94°C for 45 seconds, 50°C for 45 seconds and 72°C for 2 minutes with 5 U of Vent polymerase (New England Biolabs, Beverly, Mass.). The PCR product was digested with EcoRI and HindIII and cloned into pBluescript SK-, restriction mapped and sequenced in its entirety. The gene encoding the M antigen was deposited in GenBank, and its accession number is AF026268.

20 **Results**

M antigen amino acid sequencing. Peptides sequences of the M antigen were determined after digestion of purified M glycoprotein with lysyl endopeptidase, and purification using high-performance liquid chromatography (HPLC). Undigested antigen and internal peptides were sequenced by Edman degradation. The amino acid sequences of the NH₂ terminus and 6 internal peptides are shown in Table 1. The amino acid sequences of two internal

peptides, V22 and V18, of the M protein (Table 1) showed 30 66-73% of identity with sequences of catalases of *Schizosaccharomyces pombe* (gpD55675 YSPC_1) and *Aspergillus niger* (gpZ23138 ANCATRGNA_1).

Cloning and sequencing of the M gene. The significant degree of homology of the two internal peptides V22 and V18 to fungi catalases suggested a certain arrangement in the protein. Considering their positions, two degenerate oligonucleotides (sense primer M4F and antisense primer M8R) were designed, based upon the two internal peptides V22 and V18, respectively, and used in a PCR reaction to amplify a 320 base pair fragment of *H. capsulatum* genomic DNA. A 300 base pair PCR product was achieved using M4F and M8R as primers, and confirmed by Southern blot to represent a unique gene of *H. capsulatum*. Sequence analysis of this 300 base pair amplicon obtained by the dideoxy chain terminator method enclosed the two native internal peptides, confirming that the PCR product encoded a region of the gene encoding the M antigen.

To isolate the entire gene encoding the M antigen, the 300 base pair PCR fragment was gel purified in 1% agarose, and used to screen an *H. capsulatum* genomic DNA library. A *Bam*H1 genomic fragment of 4.0 kb carrying the gene encoding the M antigen was isolated and characterized. This fragment was subcloned into pBluescript II KS, and was sequenced in its entirety in both directions. SEQ ID NO:1 shows the complete nucleotide sequence of the *H. capsulatum* gene encoding the M antigen, and SEQ ID NO:2 shows the deduced amino acid sequence, which consisted of 707 amino acid residues (including a sixteen amino acid leader sequence) with an estimated molecular weight of about 78,172 Da.

The coding region of the M antigen gene is set forth in SEQ ID NO:1. It is interrupted by 5 introns, which begin and end at the base pair numbers 794-851, 1078-1167, 1584-1705, 1871-1949 and 2125-2207, with the

5' and 3' extremities presenting the GT/AG consensus. The 5'-565 base pair flanking sequence of this gene (the 565-base pair sequence directly preceding the first exon (first coding sequence)) exhibited similarity with the 5 promoter regions of eucaryotic genes. A TATA element is present at base pair position 318, and a T+C-rich pyrimidine block is found downstream at base pair position 365. The CAA motif is found twice upstream of the T+C block at base pair positions 34 and 341. The 3'-region downstream from the M antigen gene open reading frame contains a pentanucleotide (5'-AAATA-3') at base pair position 3134, 19 nucleotides downstream from the termination codon. This sequence is similar to the polyadenylation consensus sequence described in 10 eukaryotic organisms. It may play a role in the 15 termination of transcription, processing, and addition of poly(A) at the 3'-terminus.

20 **Protein structure.** Sequencing of the N-terminus of the native protein revealed that the first residue of the mature protein is the serine residue at base pair position 566. The mature protein is 691 amino acids with a predicted size of 76,398 Da. Therefore, the expected M antigen gene has a leader peptide composed of 16 amino acids (the 16 amino acids which precede the serine 25 residue at base pair number 566, and which begin with methionine) resulting in an amino acid sequence of 707 amino acids. Five potential N-glycosylation sites (NXT or NXS) were predicted.

30 **Comparison of the amino acid sequence of the M antigen gene with known sequences.** The earlier data base results showing that two peptides sequences of M protein had 66-73% of identity with sequences of catalases of

100-000000-0000000000

5 *Schizosaccharomyces pombe* (gpD55675 YSPC_1) and
Aspergillus niger (gpZ23138 ANCATRGNA_1) suggested that
the M antigen could be a catalase. Comparison of the M
deduced amino acid sequence with known fungal catalases
from *Aspergillus fumigatus* (GenBank accession number
u87850), *Eimericella nidulans* (GenBank accession number
u80672), *Aspergillus niger* (GenBank accession number
l15474), and *Saccharomyces cerevisiae* (GenBank accession
number x13028), using a Genetics Computer Group, Inc..
10 computer program, demonstrated 61.2, 60.4, 53.2 and 21.7%
of similarity at the amino acid level, respectively. The
M antigen amino acid sequence can be divided into parts
of high and low homology with these other amino acid
sequences, which may suggest functional domains.

15 Copy number of M gene. Southern blot of *H.*
capsulatum genomic DNA digested with various restriction
enzymes was probed with the 320-base pair PCR product in
order to evaluate the genomic organization of the M
antigen gene. A single hybridized band of 4.0 kb was
seen with the *Bam*HI-digested genomic DNA, which
corresponded to the size of the lGem11 purified inserts.
20 The hybridization profile of the other fragments
manifested only a single band, suggesting that a single
copy or few copies of the M antigen gene could occur in
25 the genome.

30 The foregoing Example is provided to enable one of
ordinary skill in the art to practice the present
invention. This example is merely illustrative, however,
and should not be read as limiting the scope of the
invention as it is claimed in the appended claims.

While the present invention has been described
herein with some specificity, and with reference to

certain preferred embodiments thereof, those of ordinary skill in the art will recognize numerous variations, modifications and substitutions of that which has been described which can be made, and which are within the
5 scope and spirit of the invention. It is intended that all of these modifications and variations be within the scope of the present invention as described and claimed herein, and that the invention be limited only by the scope of the claims which follow, and that such claims be
10 interpreted as broadly as is reasonable.

Specific nucleic acids, antigens, antibodies, vaccines, methods and kits within the scope of the invention include, but are not limited to, the nucleic acids, antigens, antibodies, vaccines, methods and kits described herein. Contemplated equivalents of the nucleic acids, antigens, antibodies, vaccines, methods and kits described herein include nucleic acids, antigens, antibodies, vaccines, methods and kits which otherwise correspond thereto, and which have the same general properties thereof, wherein one or more simple variations are made which do not adversely affect the function of the nucleic acids, antigens, antibodies, vaccines, methods and kits as described herein.

The Sequence Listing which is present herein uses
25 the symbols for bases and amino acids which are described in §2423 of the U.S. Patent and Trademark Office Manual of Patent Examining Procedure, in which R represents A or G, Y represents C or T/U and V represent A or C or G.

Throughout this application, various patents,
30 publications, books, nucleic acid and amino acid sequences, and computer programs have been cited. The

entireties of each of these patents, publications, books, nucleic acid and amino acid sequences, and computer programs are hereby incorporated by reference herein into this application.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Zancope-Oliveia, Rosely M.
et al.,

5 (ii) TITLE OF INVENTION: Nucleic Acids of the M Antigen
Gene of Histoplasma Capsulatum, Isolated and
Recombinantly-Produced
Antigens, Vaccines and Antibodies, Method

(iii) NUMBER OF SEQUENCES: 13

10 (iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: Fitch, Even, Tabin & Flannery
- (B) STREET: 135 South LaSalle Street, Suite 900
- (C) CITY: Chicago
- (D) STATE: IL
- (E) COUNTRY: USA
- (F) ZIP: 60603-4277

15 (v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Diskette
- (B) COMPUTER: IBM Compatible
- (C) OPERATING SYSTEM: Windows
- (D) SOFTWARE: FastSEQ for Windows Version 2.0

20 (vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- (C) CLASSIFICATION:

25 (vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:

30 (viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Kaba, Richard A
- (B) REGISTRATION NUMBER: 30,562
- (C) REFERENCE/DOCKET NUMBER: 6314/62527

35 (ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 312-372-7842
- (B) TELEFAX: 312-372-7848
- (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3862 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Histoplasma capsulatum
- (B) STRAIN: var. capsulatum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGATCC TGCT GGCTCCGATA ACTTTGCTT ATCCAAGGGT CTCGGCGAAT GCCAGGTGCC
ATCGATCTAT ATTTGAAGT TTATCACCTC AATGGCTTCA CCCCATGACG CACCTTTAT
50 TTTTATTTTC ATTCACTTTC TCTGTGGCAA ACATGCAGGT ATGCGAGCTC TGGACCCTGG

60

120

180

	GGTGTGGCCC	TTGATGCATA	TGGTTTATT	ATAGCCGCC	GGAAGCCCTG	GCCTGTTAAA	240
	TTTGGACCT	CCTCCCGCCA	TCCTTCAA	ACTTCGTGCG	TCGTTTCCC	ATTCCCCCCC	300
	TCCCCATTG	GGTCCCSTAT	AGGCCACTGC	GTGCTCACT	CAAGAAGGGT	CCCAGTCAT	360
	TTGGTCCCTA	CCCTCTCAA	CACTATCTGC	ATATGTAATA	TATATCGATA	TCTAACATGCC	420
5	ATTGATTATT	TGTCTCTTC	AGCATCTTT	TGTCTCGAGC	AAGCTTACTC	CACGTTCAAT	480
	TCAGGGGTA	AAAATGCGGT	CGCTCAAGCT	TATACTCGCC	TCGGCGGGTG	TTGTTTCTGC	540
	AGCCTGTCCC	TACATGTCAG	GGGAGATGCC	TAGCGGTCA	AAAGGCCCCC	TCGATGCCG	600
	CCATGACACT	CTCTCCGACC	CTACGGACCA	GTTTCTTAGC	AAGTTTACA	TTGACGATGA	660
	ACAGTGGTG	CTAACACCGG	ACGTGGTGG	TCCCATCGG	GACCAACACA	GCCTGAAGGC	720
10	TGGAAATAGA	GGCCCAACTC	TACTTGAGGA	TTTATCTTC	CGCGAGAAGA	TTCAACACTT	780
	TGATCATGAG	AGGGTATGTA	GATACAAAAT	ATGTGACCGT	GTGCAAATC	CGCTAATTCA	840
	ATTTTACGCA	GGTTCTGAG	CGCGCCGTCC	ATGCTCGAGG	AGCTGGTGC	CATGGCGTAT	900
	TCACATCTA	TAATAACTGG	TCGAATATCA	CAGCCGATC	CTTCTTGAAC	GCGGCAGGAA	960
	AGCAGACACC	AGTATTCTGT	GGGTTTCTC	CAGTCGTG	TAGCAGAGGC	AGTGTGACT	1020
15	CTGTCGCGA	TATCCACCGG	GTGGCACCC	GTCTGTATC	CGATGAAGGC	AATTTGGTA	1080
	AGCATTATAT	CGTGGTAGTC	ATACTCTATA	CAGCACAAACA	AATATGAAATA	CAAACCCAGG	1140
	ACCTAGGCTG	ACTACTCGGC	AATGTAGATA	TCGTCGAAA	CAACGTTCCA	GTCTTCTCA	1200
	TTCAGGACGC	TATTCATTTC	CCTGATTITGA	TTCACGCTGT	CAAGCCGCAA	CCAGACAGTG	1260
	AAATCCCCA	GGCTGCAACT	GCACATGATA	CGGCATGGGA	TTCCTCAGC	CAGCAGCCCA	1320
20	GCTCATTCGA	TGCCCTCTTC	TTGGCAATGT	CAGGACATGG	AATCCCTCGC	TCAATGCGTC	1380
	ATGTTGATGG	GTGGGGCGTC	CATACTTTC	GACTTGTAC	CGACGAGGGC	AACTCGACT	1440
	TGGTCAAGTT	TCGCTGGAAG	ACCCCTCAAAG	GAAGAGCGGG	CCTGGTATGG	GAAGAGGCAC	1500
	AGGCTCTGG	CGGAAAAGAT	CCGACTTCC	ATCGACAAAGA	CCTCTGGGAT	GCCATTGAAT	1560
	CTGGAAGTA	CCCTGAGTGG	GAGGTAAGAT	ATGATTCCCC	CAAATCTTAA	GTTCTGACAG	1620
25	TGTTTCTCTG	CTCTGCGGT	IGCTCTTTTC	GTCTTTTCT	ATATCTTCAA	CTAAGACTGA	1680
	CTTTATATAC	GTTTTACTCA	TATAGCTGGG	CTTCAATTG	GTGAATGAAG	CAGATCAATC	1740
	CAAGTTTGT	TCGATCTAT	TAGATCCCAC	CAAATCATC	CCAGAAGAAC	TTGTTCTTT	1800
	CACCCCAATC	GGAAAAATGG	TCTTGAACCG	AAACCCAAA	AGTATTITG	CCGAAACTGA	1860
	GCAGATCATG	GTTGTCAC	CCCCATATA	TTTGGATAT	GAATACATGT	ATAGCTAGAT	1920
30	GAAGCGTATA	TCTAAATAT	TTTCCACAGT	TCCAACCAGG	TCACTGTAGT	CGCGGAATTCG	1980
	ATTCACCGA	TGACCCCTTTC	CTTCAGGGCC	GCTTGTACTC	CTACCTTGC	ACTCAATTGA	2040
	ATCGCCATGG	AGGTCCAAC	TTCGAGCAAC	TGCCGATCAA	CAGACCCCGC	ATCCCATTCC	2100
	ATAACAACAA	TCGCGACGGT	GCTGGTAAGC	TACTTCTCAC	CTACCATGTC	AACTTCCATC	2160
	TTGACCCAAAT	CGATTCTGAT	AGAGTATTAA	CATCCCCGTC	TGACACAGGAC	AAATGTTCAT	2220
35	CCCTCTAAC	ACGGCGCAT	ATACACCCAA	CTCAATGAGC	AACGGATTCC	CACAACAAAGC	2280
	CAACCGGACC	CATAACAGAG	GATTCTTCAC	CGCACCTGGG	CGTATGGTAA	ATGGACCACT	2340
	AGTGCAGGAG	CTCAGCCCGA	GCTTCAACGA	CGTCTGGTCC	CAACCGCGTC	TCTTCTACAA	2400
	CTCACTCACG	GTCTTCGAGA	AGCAATTCTC	CGTCAACGCC	ATGCCCTTCG	AAAACCTCCA	2460
	CGTGCAGGAGT	GAAACCGTGC	CTAAGAACGCT	CATCATCCG	CTGAACCCGG	TGCAAAACCGA	2520
40	CCTGCCCGC	CGCGTCGCGC	TAGCTATCGG	CGTCAACGCC	CCATCCCCGG	ACCCAACCTT	2580
	CTACCCAAAC	AAGGCCAACG	TCCCCATCGG	CACCTTCGGC	ACGAATCTCC	TGCGGCTCGA	2640
	CGGGCTGAAA	ATGCCCTCTCC	TGACAAGAGA	CGACGGTAGC	TTCACGATCG	CGGAGCAGCT	2700
	CCGGGCCGCG	TTTAACACGG	CCAACAAACAA	AGTAGATATC	GTCCTAGTGG	GCTCATCCGT	2760
	TGATCCCCAA	CGCGCGTGC	ACATGACCTA	TTCCGGCGC	GACGGCTCGA	TCTTCGATGTC	2820
45	CGTATCGTC	GTGGCGGCC	GTCTCACGAG	CGCCTCAACG	CAATACCCAA	GAGGTCGCC	2880
	GCTCAGGATT	ATTACGGATG	CATACCGTA	TGGAAAGCCC	GTTGGCGCCG	TCGGTGACGG	2940
	TAGCAATGAA	GCCCTTCGTG	ACGTCCTTAT	GGCCGCTGGT	GGGGATGCGT	CGAATGGGCT	3000
	GGACCGACCC	GGTGTGTATA	TTTCAACCGA	TGTGAGTGG	CCCTACGTTA	GAAGTGTCTT	3060
	GGACGGATTG	ACGGCATATC	GGTTCTTGG	TCGGTTCCCG	TTGGATAGAA	GCTTGGTATG	3120
50	AGGTTGGGG	CGCAAAATG	GGTTTACTGA	CCCCCCCCCC	CCCTTTTTT	TTTCCCTTT	3180
	CTGTTTTTCC	ATCTTGGTT	GAGGTAAATA	TGCAGATATC	AGTAATTGTC	GTTTACGAAA	3240
	GCCGGTGTCA	AGCTTCANGA	GGCCTAATT	ATTGAAAGAG	GAGCTGAAAG	TGAAATCTG	3300
	GTGTAACAT	AATAATTAT	AATAACTAA	AACTTATAAT	TAATGTCAT	TGTAATTTC	3360
	TCTCACATT	AATCTATATA	TGATCTTGT	CCTTTCTGAG	TGTGTTAG	TAAGCCAAGA	3420
55	GAGACAAATA	ATGATAGATT	AACAAATAAT	TGCACACCA	ATAGGCCTTC	CCTCACGATA	3480
	TCAGATATTA	TCTATCATGT	TGTAATGATA	CCTCAAAAT	GCCACAAGCT	TGCTGTATAT	3540
	TGAATATTAA	TATGCTGTAA	ATGTAGGGAA	GAGCGTACCA	TCCAAATAAC	CAGAAAAAAC	3600
	TGTTTCTAGT	TAATACTCTCA	CTAAGGTCCG	TCGTCGTCTAT	TTGAATGGC	TGCGGCAAGC	3660
	TGACTATCTG	ATAAAATG	CTGTATTCTC	GCTTCACGAC	GCAATGTTATG	ACTTTCGAAT	3720
60	ATAGATAAAA	CCTGAACGAT	TTAGCCCCCTG	TTGGGGAAA	TAGGGGTTAG	GGGGCGAGC	3780
	TACATATCAT	TCCCATATGA	CCAAAAACTA	AAATAGATAT	ATATATATAT	ATATATATAT	3840
	ACAACACCTT	CAAAAAGGAT	CC				3862

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 707 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

	Met Pro Ser Gly Gln Lys Gly Pro Leu Asp Arg Arg His Asp Thr Leu	
	1 5 10 15	
5	Ser Asp Pro Thr Asp Gln Phe Leu Ser Lys Phe Tyr Ile Asp Asp Glu	
	20 25 30	
	Gln Ser Val Leu Thr Thr Asp Val Gly Gly Pro Ile Glu Asp Gln His	
	35 40 45	
10	Ser Leu Lys Ala Gly Asn Arg Gly Pro Thr Leu Leu Glu Asp Phe Ile	
	50 55 60	
	Phe Arg Gln Lys Ile Gln His Phe Asp His Glu Arg Val Pro Glu Arg	
	65 70 75 80	
	Ala Val His Ala Arg Gly Ala Gly Ala His Gly Val Phe Thr Ser Tyr	
	85 90 95	
15	Asn Asn Trp Ser Asn Ile Thr Ala Ala Ser Phe Leu Asn Ala Ala Gly	
	100 105 110	
	Lys Gln Thr Pro Val Phe Val Arg Phe Ser Thr Val Ala Gly Ser Arg	
	115 120 125	
	Gly Ser Val Asp Ser Ala Arg Asp Ile His Gly Phe Ala Thr Arg Leu	
	130 135 140	
20	Tyr Thr Asp Glu Gly Asn Phe Asp Ile Val Gly Asn Asn Val Pro Val	
	145 150 155 160	
	Phe Phe Ile Gln Asp Ala Ile Gln Phe Pro Asp Leu Ile His Ala Val	
	165 170 175	
25	Lys Pro Gln Pro Asp Ser Glu Ile Pro Gln Ala Ala Thr Ala His Asp	
	180 185 190	
	Thr Ala Trp Asp Phe Leu Ser Gln Gln Pro Ser Ser Leu His Ala Leu	
	195 200 205	
	Phe Trp Ala Met Ser Gly His Gly Ile Pro Arg Ser Met Arg His Val	
	210 215 220	
30	Asp Gly Trp Gly Val His Thr Phe Arg Leu Val Thr Asp Glu Gly Asn	
	225 230 235 240	
	Ser Thr Leu Val Lys Phe Arg Trp Lys Thr Leu Gln Gly Arg Ala Gly	
	245 250 255	
35	Leu Val Trp Glu Glu Ala Gln Ala Leu Gly Gly Lys Asn Pro Asp Phe	
	260 265 270	
	His Arg Gln Asp Leu Trp Asp Ala Ile Glu Ser Gly Arg Tyr Pro Glu	
	275 280 285	
	Trp Glu Leu Gly Phe Gln Leu Val Asn Glu Ala Asp Gln Ser Lys Phe	
	290 295 300	
40	Asp Phe Asp Leu Leu Asp Pro Thr Lys Ile Ile Pro Glu Glu Leu Val	
	305 310 315 320	
	Pro Phe Thr Pro Ile Gly Lys Met Val Leu Asn Arg Asn Pro Lys Ser	
	325 330 335	
45	Tyr Phe Ala Glu Thr Glu Gln Ile Met Phe Gln Pro Gly His Val Val	
	340 345 350	
	Arg Gly Ile Asp Phe Thr Asp Asp Pro Leu Leu Gln Gly Arg Leu Tyr	
	355 360 365	
	Ser Tyr Leu Asp Thr Gln Leu Asn Arg His Gly Gly Pro Asn Phe Glu	
	370 375 380	
50	Gln Leu Pro Ile Asn Arg Pro Arg Ile Pro Phe His Asn Asn Asn Arg	
	385 390 395 400	
	Asp Gly Ala Gly Gln Met Phe Ile Pro Leu Asn Thr Ala Ala Tyr Thr	
	405 410 415	
55	Pro Asn Ser Met Ser Asn Gly Phe Pro Gln Gln Ala Asn Arg Thr His	
	420 425 430	
	Asn Arg Gly Phe Phe Thr Ala Pro Gly Arg Met Val Asn Gly Pro Leu	
	435 440 445	
	Val Arg Glu Leu Ser Pro Ser Phe Asn Asp Val Trp Ser Gln Pro Arg	
	450 455 460	
60	Leu Phe Tyr Asn Ser Leu Thr Val Phe Glu Lys Gln Phe Leu Val Asn	
	465 470 475 480	
	Ala Met Arg Phe Glu Asn Ser His Val Arg Ser Glu Thr Val Arg Lys	
	485 490 495	
65	Asn Val Ile Ile Gln Leu Asn Arg Val Asp Asn Asp Leu Ala Arg Arg	
	500 505 510	
	Val Ala Leu Ala Ile Gly Val Glu Pro Pro Ser Pro Asp Pro Thr Phe	
	515 520 525	
	Tyr His Asn Lys Ala Thr Val Pro Ile Gly Thr Phe Gly Thr Asn Leu	
	530 535 540	
70	Leu Arg Leu Asp Gly Leu Lys Ile Ala Leu Leu Thr Arg Asp Asp Gly	

545 550 555 560
 Ser Phe Thr Ile Ala Glu Gln Leu Arg Ala Ala Phe Asn Ser Ala Asn
 565 570 575 580
 Asn Lys Val Asp Ile Val Leu Val Gly Ser Ser Leu Asp Pro Gln Arg
 580 585 590 595
 Gly Val Asn Met Thr Tyr Ser Gly Ala Asp Gly Ser Ile Phe Asp Ala
 595 600 605 610
 Val Ile Val Val Gly Gly Leu Leu Thr Ser Ala Ser Thr Gln Tyr Pro
 610 615 620 625
 Arg Gly Arg Pro Leu Arg Ile Ile Thr Asp Ala Tyr Ala Tyr Gly Lys
 625 630 635 640
 Pro Val Gly Ala Val Gly Asp Gly Ser Asn Glu Ala Leu Arg Asp Val
 645 650 655 660
 Leu Met Ala Ala Gly Gly Asp Ala Ser Asn Gly Leu Asp Gln Pro Gly
 660 665 670 675
 Val Tyr Ile Ser Asn Asp Val Ser Glu Ala Tyr Val Arg Ser Val Leu
 675 680 685 690
 Asp Gly Leu Thr Ala Tyr Arg Phe Leu Asn Arg Phe Pro Leu Asp Arg
 690 695 700 .
 20 Ser Leu Val
 705

(2) INFORMATION FOR SEQ ID NO:3:

- 25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 8 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

30 Ser Asp Pro Thr Asp Gln Phe Leu
 1 5

(2) INFORMATION FOR SEQ ID NO:4:

- 35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

40 Asp Phe Ile Phe Arg Gln Lys Ile Gln His Phe Asp His Glu Arg
 1 5 10 15

(2) INFORMATION FOR SEQ ID NO:5:

- 45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 9 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

50 Thr Leu Gln Gly Arg Ala Gly Leu Val
 1 5

(2) INFORMATION FOR SEQ ID NO:6:

- 55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 16 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: None
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Gln Ala Leu Gly Gly Lys Asn Pro Asp Phe His Arg Gln Asp Leu
1 5 10 15

5 (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: None

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ser Gly Arg Tyr Pro Glu
1 5

15 (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: None

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Phe Asp Phe Asp Leu Leu Asp Pro Thr Lys
1 5 10

25 (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ile Ile Pro Glu Glu Leu Val Pro Phe Thr Pro Ile Gly Lys
1 5 10

35 (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Other
(ix) FEATURE:

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AARAAYCCVG AYTTY

15

45 (2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Other

(ix) FEATURE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TTNCCDATNG TRAA

14

(2) INFORMATION FOR SEQ ID NO:12:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGGAATCCTC CGACCCTACG GA

22

(2) INFORMATION FOR SEQ ID NO:13:

15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ACCAAGCTTC TATCCAACGG GAACCGA

27

WHAT IS CLAIMED IS:

1. An isolated nucleic acid specific to
Histoplasma capsulatum comprising:
a nucleic acid having a nucleotide sequence as set
forth in the Sequence Listing as SEQ ID NO:1;
5 a nucleic acid having a nucleotide sequence which is
complementary to the nucleotide sequence set forth in the
Sequence Listing as SEQ ID NO:1;
a nucleic acid which is substantially the same as a
10 nucleic acid having a nucleotide sequence as set forth in
the Sequence Listing as SEQ ID NO:1;
a nucleic acid which is substantially the same as a
nucleic acid having a nucleotide sequence which is
complementary to the nucleotide sequence set forth in the
15 Sequence Listing as SEQ ID NO:1;
a fragment of a nucleic acid having a nucleotide
sequence as set forth in the Sequence Listing as SEQ ID
NO:1;
20 a fragment of a nucleic acid having a nucleotide
sequence which is complementary to the nucleotide
sequence set forth in the Sequence Listing as SEQ ID
NO:1;
a fragment of a nucleic acid which is substantially
25 the same as a nucleic acid having a nucleotide sequence
as set forth in the Sequence Listing as SEQ ID NO:1; or
a fragment of a nucleic acid having a nucleotide
sequence which is substantially the same as a nucleic
acid which is complementary to the nucleotide sequence
set forth in the Sequence Listing as SEQ ID NO:1;
30 wherein the nucleic acid does not contain the
nucleotide sequences 5' CGAAGTCGAGGCTTCAGCATG3' ,
5' TATTAGCTCTAGAATTACCACGGGTATCCAAGTAGTAAGG3' ,
5' CCCCGAAGGGCATTGGTTTTATCTAATAATACACCCCC3' , or
nucleotide sequences complementary thereto,
35 and wherein the nucleic acid is not a nucleic acid

consisting essentially of between 10 and 100 nucleotides
which is able to form a hybrid at 60°C with a nucleotide
polymer having a nucleotide sequence of
5 CGAAGTCGAGGCTTCAGCATG3 , 5 CATGCTGAAAGCCTCGACTTCG3 ,
5 CAUGCUGAAAGCCUCGACUUCG3 or 5 CGAACGAGGCUUUCAGCAUG3 .

2. The nucleic acid of Claim 1, wherein the nucleic
acid has the nucleotide sequence set forth in the
Sequence Listing as SEQ ID NO:1.

3. The nucleic acid of Claim 1, wherein the nucleic
10 acid has a nucleotide sequence which is complementary to
the nucleotide sequence set forth in the Sequence Listing
as SEQ ID NO:1.

4. The nucleic acid of Claim 1, wherein the nucleic
acid has a nucleotide sequence which is substantially the
15 same as a nucleic acid having a nucleotide sequence as
set forth in the Sequence Listing as SEQ ID NO:1.

5. The nucleic acid of Claim 1, wherein the nucleic
acid has a nucleotide sequence which is substantially the
same as a nucleic acid having a nucleotide sequence which
20 is complementary to the nucleotide sequence set forth in
the Sequence Listing as SEQ ID NO:1.

6. The nucleic acid of Claim 1, wherein the nucleic
acid is a fragment of a nucleic acid having a nucleotide
sequence as set forth in the Sequence Listing as SEQ ID
25 NO:1.

7. The nucleic acid of Claim 1, wherein the nucleic
acid is a fragment of a nucleic acid having a nucleotide
sequence which is complementary to the nucleotide
sequence set forth in the Sequence Listing as SEQ ID
30 NO:1.

8. The nucleic acid of Claim 1, wherein the nucleic acid is a fragment of a nucleic acid having a nucleotide sequence which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

9. The nucleic acid of Claim 1, wherein the nucleic acid is a fragment of a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

10. An isolated or recombinantly-produced antigen specific to *Histoplasma capsulatum* comprising:

15 a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1;

20 a fragment of a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1;

25 a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1; or

30 a fragment of a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

11. The antigen of Claim 10, wherein the antigen is a polypeptide encoded by a nucleic acid having a

nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

12. The antigen of Claim 11, wherein the antigen
5 has an amino acid sequence as set forth in the Sequence Listing as SEQ ID NO:2.

10 13. The antigen of Claim 10, wherein the antigen is a fragment of a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to

the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

15 14. The antigen of Claim 10, wherein the antigen is a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

20 15. The antigen of Claim 10, wherein the antigen is a fragment of a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

25 16. A vector comprising a nucleic acid specific to *Histoplasma capsulatum*, wherein the nucleic acid:

has a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1;

30 has a nucleotide sequence which is substantially the same as a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1;

is a fragment of a nucleic acid having a nucleotide

sequence as set forth in the Sequence Listing as SEQ ID NO:1; or

is a fragment of a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1;
and wherein the vector is suitable for expressing the nucleic acid.

17. The vector of Claim 16, wherein the nucleic acid has a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

18. The vector of Claim 16, wherein the nucleic acid has a nucleotide sequence which is substantially the same as a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

19. The vector of Claim 16, wherein the nucleic acid is a fragment of a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

20. The vector of Claim 16, wherein the nucleic acid is a fragment of a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

21. A method for detecting a previous or current *Histoplasma capsulatum* infection in a subject, comprising:

(a) contacting a fluid or tissue sample from the subject which contains antibodies with an isolated or recombinantly-produced antigen which is specific to *Histoplasma capsulatum*; and

(b) detecting the presence of binding between the antibodies and the antigen, the presence of binding indicating the presence of a previous or current *Histoplasma capsulatum* infection in a subject,

wherein the antigen is:

a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1;

a fragment of a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1;

a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1; or

a fragment of a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

22. The method of Claim 21, wherein the antigen is a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

23. The method of Claim 22, wherein the antigen has an amino acid sequence as set forth in the Sequence Listing as SEQ ID NO:2.

5 24. The method of Claim 21, wherein the antigen is a fragment of a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

10 25. The method of Claim 21, wherein the antigen is a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

15 26. The method of Claim 21, wherein the antigen is a fragment of a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

27. An isolated antibody produced against an antigen of Claim 10.

28. A kit for detecting a previous or current *Histoplasma capsulatum* infection in a sample comprising:

- 25 (a) an isolated nucleic acid of Claim 1, an isolated or recombinantly-produced antigen of Claim 10 or an isolated antibody of Claim 27; and
- 30 (b) instructions describing the use of the nucleic acid, antigen or antibody in the detection of a previous or current *Histoplasma capsulatum* infection.

29. The kit of Claim 28, wherein the kit contains an antigen, and the antigen is a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

30. The kit of Claim 28, wherein the kit contains an antigen, and the antigen is a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

31. A host for expressing a polypeptide specific to *Histoplasma capsulatum* comprising a vector containing a nucleic acid, wherein the vector is suitable for expressing the nucleic acid, and wherein the nucleic acid:

has a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1;

has a nucleotide sequence which is substantially the same as a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1;

is a fragment of a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1; or

is a fragment of a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

32. The host of claim 31, wherein the nucleic acid has a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

33. The host of Claim 31, wherein the nucleic acid is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

5 34. A method for detecting a past exposure to the fungus *Histoplasma capsulatum* comprising:

(a) injecting intradermally in the skin of a patient an effective amount of an isolated or recombinantly-produced antigen which is specific to

10 *Histoplasma capsulatum*; and

(b) observing the skin at the injection site at a predetermined time after injection for a presence of swelling of the skin, the presence of swelling of the skin indicating a past exposure by the patient to the

15 fungus *Histoplasma capsulatum*,

wherein the polypeptide is:

a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as

20 SEQ ID NO:1;

an antigenic fragment of a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1;

25 a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1; or

30 an antigenic fragment of a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

35. The method of Claim 34, wherein the antigen is
a polypeptide encoded by a nucleic acid having a
nucleotide sequence which is complementary to the
nucleotide sequence as set forth in the Sequence Listing
5 as SEQ ID NO:1.

36. The method of Claim 34, wherein the antigen is
an antigenic fragment of a polypeptide encoded by a
nucleic acid having a nucleotide sequence which is
complementary to the nucleotide sequence set forth in the
10 Sequence Listing as SEQ ID NO:1.

37. The method of Claim 34, wherein the antigen is
a polypeptide encoded by a nucleic acid which is
substantially the same as a nucleic acid having a
nucleotide sequence which is complementary to the
15 nucleotide sequence set forth in the Sequence Listing as
SEQ ID NO:1.

38. The method of Claim 34, wherein the antigen is
a fragment of a polypeptide encoded by a nucleic acid
which is substantially the same as a nucleic acid having
20 a nucleotide sequence which is complementary to the
nucleotide sequence set forth in the Sequence Listing as
SEQ ID NO:1.

39. A vaccine for the prevention of histoplasmosis
comprising:

- 25 (a) an effective amount of a nucleic acid of claim
1 or an isolated or recombinantly-produced antigen of
Claim 10; and
(b) a pharmaceutically-acceptable carrier.

40. A method for detecting a current *H. capsulatum* infection in a subject suspected of having an *H. capsulatum* infection comprising:

5 (a) contacting a fluid or tissue sample from the subject which contains antigens with antibodies generated against an antigen which contains an epitope which is unique to *H. capsulatum*; and

10 (b) detecting the presence of binding between the antigens and the antibodies, the presence of binding indicating the presence of a current *H. capsulatum* infection in the subject,

wherein the antigen is:

15 a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1;

20 a fragment of a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1;

25 a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1; or

30 a fragment of a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

NUCLEIC ACIDS OF THE M ANTIGEN GENE OF
HISTOPLASMA CAPSULATUM, ISOLATED AND RECOMBINANTLY-
PRODUCED ANTIGENS, VACCINES AND ANTIBODIES,
METHODS AND KITS FOR DETECTING HISTOPLASMOSIS

ABSTRACT OF THE DISCLOSURE

The present invention provides the nucleotide sequence of the M antigen gene of *H. capsulatum*, which is set forth in the Sequence Listing as SEQ ID NO:1, a nucleic acid having the nucleotide sequence complementary thereto, and nucleic acids having a nucleotide sequence which is substantially the same as the foregoing nucleotide sequences. The present invention also provides vectors and host expression systems containing the foregoing nucleic acids, and isolated or recombinantly-produced antigens encoded by the foregoing nucleic acids. The present invention further provides antibodies generated against the foregoing antigens, and methods and kits for detecting a previous or current *Histoplasma capsulatum* infection in a subject, and for diagnosing histoplasmosis.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Zancope-Oliveia, Rosely M.
et al.,

5 (ii) TITLE OF INVENTION: Nucleic Acids of the M Antigen
Gene of *Histoplasma capsulatum*, Isolated and
Recombinantly-Produced
Antigens, Vaccines and Antibodies, Method

(iii) NUMBER OF SEQUENCES: 13

10 (iv) CORRESPONDENCE ADDRESS:
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(F) ZIP: 60603-4277

15 (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: Windows
(D) SOFTWARE: FastSEQ for Windows Version 2.0

20 (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

25 (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

30 (viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3862 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Genomic DNA
(vi) ORIGINAL SOURCE:
(A) ORGANISM: *Histoplasma capsulatum*
(B) STRAIN: var. capsulatum

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGATCCTGCT GGCTCCGATA ACTTTGCTTT ATCCAAGGGT CTCGGCGAAT GCCAGGTGCC	60
ATCGATCTAT ATTTGAAGT TTATCACCTC AATGGCTTCA CCCCATGACG CACCTTTAT	120
50 TTTTATTTTC ATTCACTTTC TCTGTGGCAA ACATGCAGGT ATGCGAGCTC TGGACCCCTGG	180

	GGTGTGGGCC	TTGATGCATA	TGGTTTATT	ATAGCCGCC	GGAAGCCCTG	GCCTGTTAAA	240
	TTTGGACCT	CCTCCGCCA	TTCTTCCAA	ACTTCGTGCG	TCCGTTCCC	ATTCCCCCCC	300
	TCCCCATTG	GGTCCCTAT	AGGCCACTGC	GTGCTCCACT	CAAGAAGGGT	CCCAGTCAT	360
	TTGGTCCTA	CCCTCTCAA	CACTATCTGC	ATATGTAATA	TATATCGATA	TCTAACCTGCC	420
5	ATTGATTATT	TGTCTCTTC	AGCATCTTT	TGTCTCGAGC	AAGCTTACTC	CACGTTCAAT	480
	TCAGGGGTA	AAAATGCGGT	CGCTCAAGCT	TATACTCGCC	TCGGCGGGTG	TTGTTTCTGC	540
	AGCCTGCCC	TACATGTAG	GGGAGATGCC	TAGCGGTAG	AAAGGCCCCC	TCGATGCCG	600
	CCATGACACT	CTCTCCGACC	CTACGGACAA	GTTCCTTAGC	AGTTTTTACA	TTGACGATGA	660
	ACAGTCGGT	CTAACAAACGG	ACGTTGGTGG	CCCCATCGAG	GACCAACACA	GCCTGAAGGC	720
10	TGAAATAGA	GGCCCAACTC	TACTTGAGGA	TTTTATCTTC	CGCCAGAAGA	TTCAACACTT	780
	TGATCATGAG	AGGGTATGTA	GATACAAAAT	ATGTGACCCT	GTGCAAATC	CGCTAAATTCA	840
	ATTTACGCA	GGTTCTGAG	CGCGCCGTCC	ATGCTCGAGG	AGCTGGTGC	CATGGCGTAT	900
	TCACATCTA	TAATACTGC	TCGAATCATC	CAGCCGCATC	TTCTCTGAA	GCGGCAGGAA	960
	AGCAGACACC	AGTATTCGTG	CGGTTTCTA	AGTCGCTGG	TAGCAGAGGC	AGTGGTGA	1020
15	CTGCTCGGA	TATCCACGGG	TTTGACGACC	GTCTGTATAC	CGATGAAAGG	AATTTGGTA	1080
	AGCATTATAT	CGTGGTAGTC	ATACTCATAA	CAGCACAACA	AATATGAATA	CAAACCCAGG	1140
	ACCTAGGCTG	ACTACTCGGC	AATGTAGATA	TCGTCGGAAA	CAACGTTCCA	GTCTCTTC	1200
	TTCAGGACG	TATTCAGTAT	CTCTGATTG	TTCACTGCTG	CAAGACGCGA	CCAGACAGTG	1260
	AAATTCCCCA	GGCTGCAACT	GCACATGATA	CCGGCATGGG	TTTCTCTAGC	CAGCAGCCCA	1320
20	GCTATTGCA	TGCCCTCTTC	TGGGCAATGT	CAGGACATGG	AATCCCTCGC	TCAATGCGTC	1380
	ATGTTGATGG	GTGGGGCGTC	CATACCTTCC	GACTTGTAC	CGACGAGGGC	AACTCGACCT	1440
	TGGTCAAGTT	TCGCTGGAAG	ACCCCTCAAAG	GAAGAGCGGG	CCTGGTATGG	GAAGAGGCAC	1500
	AGGCTCTGG	CGGAAAGAATGG	CCGACTTCC	ATCGACAAAGA	CCTCTGGGAT	GCCATTGAAT	1560
	CTGGAAGGT	CCCTGAGTGG	GAGGTAAGAT	ATGATCCCC	CAAATCATT	GTTCTGACAG	1620
25	TGTTCTCTG	CTCTGTCGGT	TGCTCTTTTC	GTCTTTTCT	ATATCTTCA	CTAACAGACTGA	1680
	CTTTATATAC	GTTTTACTCA	TATAGCTGG	CTTCAATTG	GTGAATGAAG	CAGATCAATC	1740
	CAAGTTTGAT	TCGATCTAT	TAGATCCAC	CAAATCATC	CCAGAAGAAC	TTGTTCTTT	1800
	CACCCCAATC	GGAAAAATGG	TCTTGAACCG	AAACCAAAA	AGTTATTTTG	CCGAAACTGA	1860
	GCAGATCATG	GTGGTCCAC	CCCCTATTT	TTTGAATAT	GTAAATCATGT	ATAGCTAGAT	1920
30	GAAGCGTATA	TCTAAATATA	TTTCCACAGT	TCCAACCCAG	TCATGTAGTT	CGCGGAATCG	1980
	ATTCACGGA	TGACCCCTTG	CTTCAGGGCC	GCTTGTACTC	CTACCTTGCAC	ACTCAATTGA	2040
	ATGCCCATGG	AGGTCCCAAC	TTCGAGCAAC	TGCCGATCAA	CAGACCCCGC	ATCCCATTCC	2100
	ATAAACACAA	TCGCGACGGT	GCTGGTAAGC	TACTTCTCAC	CTACCATGTC	AACTCCATC	2160
	TTGACCAAT	CGATTTGTAT	AGAGTATTA	CATCCCCGT	TCGACAGGAC	AAATGTTCAT	2220
35	CCCTCTAAC	ACGGCCGCAT	ATACACCAA	TCCAATGAGC	AACGGGATTCC	CACAACAAGC	2280
	CAACCGGACC	CATAACAGAG	GATTCTTCAC	CGCACCTGGG	CGTATGGTAA	ATGGACCACT	2340
	AGTGGCGAG	CTCAGCCCGA	GCTTCAACGA	CGTCTGGTCC	CAACCGCGTC	TCTTCTACAA	2400
	CTCACTCACG	GTCTTCGAGA	AGCAATTCT	CGTCAACGCC	ATGCGTTCG	AAAACCTCCA	2460
	CGTGGGAGT	GAACCCGTG	GTAAGAACGT	CATCATCCAG	CTGAACCGCG	TCGACAAACGA	2520
40	CCTCGCCCG	CGCGTCGCGC	TAGCTATCGG	CGTCGAACCC	CCATCCCCGG	ACCCAACCTT	2580
	CTACCAACAA	AAGGCAACCG	TCCCCATCGG	CACCTTCGGC	ACGAATCTCC	TGCGGCTCGA	2640
	CGGGCTGAAA	ATGCCCTCTCC	TGACAAGAGA	CGACGGTAGC	TTCACGATCG	CGGAGCAGCT	2700
	CCGGGCCGCG	TTAACACCG	CCAACAACAA	AGTAGATATC	GTCCTAGTGG	GCTCATCGCT	2760
	TGATCCCCA	CGCGGCGTGA	ACATGACCA	TTCCGGCGCC	GACGGCTCGA	TCTTCGATGC	2820
45	CGTGTGCGT	GTGGCGGCC	TGCTCACCGAG	CGCCTCAACAG	CAATACCCAA	GAGGTCGCC	2880
	GCTCAGGATT	ATTACGGATG	CATACGCGTA	TGGAAAGGCC	GTTGGCGCCC	TCGGTGACGG	2940
	TAGCAATGAA	GCCCTTCGTC	ACGTCTTAT	GGCCGCTGGT	GGGGATGCGT	CGAATGGCT	3000
	GGACCAGCCC	GGTGTGTATA	TTTCCAACGA	TGTGAGTGAG	GCCTACGTTA	GAAGTGTCTT	3060
	GGACGATTG	ACGGCATATC	GGTTCTTGA	TCGGTTCCC	TTGGATAGAA	GCTTGGTATG	3120
50	AGGTTGGGG	CGCAAATATG	GGTTTACTAC	CCCCCCCC	CCCTTTTT	TTTCTCTTTT	3180
	CTGTTTCTTC	ATCTTTGGTT	GAGGTAATAT	TGCAATGATC	AGTAAATTGC	GTTTACGAAA	3240
	GCCGGTGTCA	AGCTTCANGA	GGCCTAAATA	ATTGAAGAG	GAGGTTGAAG	TGAAATCTTG	3300
	GTGTAACAT	AATAATTAT	AATAACTAAT	AACTTATAAT	TAATGTCTAT	TGTAATTTC	3360
	TCTCACATT	AATCTATATT	TGATCTCTGT	CCTTGTAGC	GTGTTAAATA	TAAGCCAAGA	3420
55	GAGACAAAT	ATGATAGAT	AACAAATAAT	TGCACACCC	ATAGGCCTTC	CCTCACGATA	3480
	TCAGATATTA	TCTATCATGT	TGTAATGATA	CCTCAAAAT	GCCACAAGCT	TGCCTGATAT	3540
	TGAATATTA	TATGCTGTAA	ATGTAGGGAA	GAGCGTACCA	TCCAAATAAC	CAGAAAAACAA	3600
	TGTTTAGCT	AAAAATCTCA	CTAAGGTCGG	TCGTTGCTAT	TTGAAATGGC	TGCGGCAAGC	3660
	TGACTATCTG	ATAAAAATGT	CTGTATTCTC	GCTTCACGAC	GCATGTTATG	ACTTCGAAT	3720
60	ATAGATAAAA	CCTGAACGAT	TTAGCCCCCTG	TTGGGGGAAA	TAGGGGTTAG	GGGGCGGAGC	3780
	TACATATCAT	TCCCATATGA	CAAAAAACTA	AAATAGATAT	ATATATATAT	ATATATATAT	3840
	ACAACACCTT	CAAAAGGAT	CC				3862

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 707 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

	Met Pro Ser Gly Gln Lys Gly Pro Leu Asp Arg Arg His Asp Thr Leu		
1	5	10	15
5	Ser Asp Pro Thr Asp Gln Phe Leu Ser Lys Phe Tyr Ile Asp Asp Glu		
	20	25	30
	Gln Ser Val Leu Thr Thr Asp Val Gly Gly Pro Ile Glu Asp Gln His		
	35	40	45
10	Ser Leu Lys Ala Gly Asn Arg Gly Pro Thr Leu Leu Glu Asp Phe Ile		
	50	55	60
	Phe Arg Gln Lys Ile Gln His Phe Asp His Glu Arg Val Pro Glu Arg		
65	70	75	80
	Ala Val His Ala Arg Gly Ala Gly His Gly Val Phe Thr Ser Tyr		
	85	90	95
15	Asn Asn Trp Ser Asn Ile Thr Ala Ala Ser Phe Leu Asn Ala Ala Gly		
	100	105	110
	Lys Gln Thr Pro Val Phe Val Arg Phe Ser Thr Val Ala Gly Ser Arg		
	115	120	125
	Gly Ser Val Asp Ser Ala Arg Asp Ile His Gly Phe Ala Thr Arg Leu		
	130	135	140
20	Tyr Thr Asp Glu Gly Asn Phe Asp Ile Val Gly Asn Asn Val Pro Val		
	145	150	155
	Phe Phe Ile Gln Asp Ala Ile Gln Phe Pro Asp Leu Ile His Ala Val		
	165	170	175
25	Lys Pro Gln Pro Asp Ser Glu Ile Pro Gln Ala Ala Thr Ala His Asp		
	180	185	190
	Thr Ala Trp Asp Phe Leu Ser Gln Gln Pro Ser Ser Leu His Ala Leu		
	195	200	205
	Phe Trp Ala Met Ser Gly His Gly Ile Pro Arg Ser Met Arg His Val		
	210	215	220
30	Asp Gly Trp Gly Val His Thr Phe Arg Leu Val Thr Asp Glu Gly Asn		
	225	230	235
	Ser Thr Leu Val Lys Phe Arg Trp Lys Thr Leu Gln Gly Arg Ala Gly		
	245	250	255
35	Leu Val Trp Glu Ala Gln Ala Leu Gly Gly Lys Asn Pro Asp Phe		
	260	265	270
	His Arg Gln Asp Leu Trp Asp Ala Ile Glu Ser Gly Arg Tyr Pro Glu		
	275	280	285
	Trp Glu Leu Gly Phe Gln Leu Val Asn Glu Ala Asp Gln Ser Lys Phe		
	290	295	300
40	Asp Phe Asp Leu Leu Asp Pro Thr Lys Ile Ile Pro Glu Glu Leu Val		
	305	310	315
	Pro Phe Thr Pro Ile Gly Lys Met Val Leu Asn Arg Asn Pro Lys Ser		
	325	330	335
45	Tyr Phe Ala Glu Thr Glu Gln Ile Met Phe Gln Pro Gly His Val Val		
	340	345	350
	Arg Gly Ile Asp Phe Thr Asp Asp Pro Leu Leu Gln Gly Arg Leu Tyr		
	355	360	365
	Ser Tyr Leu Asp Thr Gln Leu Asn Arg His Gly Pro Asn Phe Glu		
	370	375	380
50	Gln Leu Pro Ile Asn Arg Pro Arg Ile Pro Phe His Asn Asn Asn Arg		
	385	390	395
	Asp Gly Ala Gly Gln Met Phe Ile Pro Leu Asn Thr Ala Ala Tyr Thr		
	405	410	415
55	Pro Asn Ser Met Ser Asn Gly Phe Pro Gin Gln Ala Asn Arg Thr His		
	420	425	430
	Asn Arg Gly Phe Phe Thr Ala Pro Gly Arg Met Val Asn Gly Pro Leu		
	435	440	445
	Val Arg Glu Leu Ser Pro Ser Phe Asn Asp Val Trp Ser Gln Pro Arg		
	450	455	460
60	Leu Phe Tyr Asn Ser Leu Thr Val Phe Glu Lys Gln Phe Leu Val Asn		
	465	470	475
	Ala Met Arg Phe Glu Asn Ser His Val Arg Ser Glu Thr Val Arg Lys		
	485	490	495
65	Asn Val Ile Ile Gln Leu Asn Arg Val Asp Asn Asp Leu Ala Arg Arg		
	500	505	510
	Val Ala Leu Ala Ile Gly Val Glu Pro Pro Ser Pro Asp Pro Thr Phe		
	515	520	525
	Tyr His Asn Lys Ala Thr Val Pro Ile Gly Thr Phe Gly Thr Asn Leu		
	530	535	540
70	Leu Arg Leu Asp Gly Leu Lys Ile Ala Leu Leu Thr Arg Asp Asp Gly		

545 550 555 560
Ser Phe Thr Ile Ala Glu Gln Leu Arg Ala Ala Phe Asn Ser Ala Asn
565 570 575
Asn Lys Val Asp Ile Val Leu Val Gly Ser Ser Leu Asp Pro Gln Arg
580 585 590
Gly Val Asn Met Thr Tyr Ser Gly Ala Asp Gly Ser Ile Phe Asp Ala
595 600 605
Val Ile Val Val Gly Gly Leu Leu Thr Ser Ala Ser Thr Gln Tyr Pro
610 615 620
10 Arg Gly Arg Pro Leu Arg Ile Ile Thr Asp Ala Tyr Ala Tyr Gly Lys
625 630 635 640
Pro Val Gly Ala Val Gly Asp Gly Ser Asn Glu Ala Leu Arg Asp Val
645 650 655
Leu Met Ala Ala Gly Gly Asp Ala Ser Asn Gly Leu Asp Gln Pro Gly
660 665 670
15 Val Tyr Ile Ser Asn Asp Val Ser Glu Ala Tyr Val Arg Ser Val Leu
675 680 685
Asp Gly Leu Thr Ala Tyr Arg Phe Leu Asn Arg Phe Pro Leu Asp Arg
690 695 700
20 Ser Leu Val
705

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

30 Ser Asp Pro Thr Asp Gln Phe Leu
1 5

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

40 Asp Phe Ile Phe Arg Gln Lys Ile Gln His Phe Asp His Glu Arg
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

50 Thr Leu Gln Gly Arg Ala Gly Leu Val
1 5

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Gln Ala Leu Gly Gly Lys Asn Pro Asp Phe His Arg Gln Asp Leu
1 5 10 15

5 (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ser Gly Arg Tyr Pro Glu
1 5

15 (2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Phe Asp Phe Asp Leu Leu Asp Pro Thr Lys
1 5 10

25 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ile Ile Pro Glu Glu Leu Val Pro Phe Thr Pro Ile Gly Lys
1 5 10

35 (2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: Other

(ix) FEATURE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AARAAYCCVG AYTTY

15

45 (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: Other

(ix) FEATURE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TTNCCDATNG TRAA

14

(2) INFORMATION FOR SEQ ID NO:12:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGGAATCCTC CGACCCTACG GA

22

(2) INFORMATION FOR SEQ ID NO:13:

15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ACCAAGCTTC TATCCAACGG GAACCGA

27

Sequence Description

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Page 1 of 3

DECLARATION FOR PATENT APPLICATION

(X) Original () Supplemental () Substitute () PCT

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled "**NUCLEIC ACIDS OF THE M ANTIGEN GENE OF HISTOPLASMA CAPSULATUM, ANTIGENS, VACCINES, AND ANTIBODIES, METHODS AND KITS FOR DETECTING HISTOPLASMOSIS,**" which is described and claimed in the specification

- (check one) [] which is attached hereto, or
 which was filed on October 26, 2000, as Application Serial No. 09/674,195 and with amendments through (if applicable), or
 in International Application No. PCT/, filed , and as amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information known by me to be material to the patentability of the claims of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code §119 (a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATIONS (ENTER BELOW IF APPLICABLE)			PRIORITY CLAIMED (MARK APPROPRIATE BOX BELOW)	
APP. NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	YES	NO
PCT/US99/09151	PCT	27 April 1999	X	

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

APPLICATION NUMBER	FILED DATE
60/083,676	30 April 1998

**DECLARATION
FOR PATENT APPLICATION**

(X) Original () Supplemental () Substitute () PCT

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APPLICATION SERIAL NO.	FILING DATE	STATUS (MARK APPROPRIATE COLUMN BELOW)		
		PATENTED	PENDING	ABANDONED

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first inventor: Rosely M. Zancope-Oliveira

Inventor's signature: _____ Date: _____

Residence: Rua Fernando Mendes No. 25/62, Copacabana, Rio De Janeiro, RJ 22021-030 Brazil

Post Office Address: Rua Fernando Mendes No. 25/62, Copacabana, Rio De Janeiro, RJ 22021-030 Brazil

Citizenship: Brazil

Full name of second inventor: Timothy J. Lott

Inventor's signature: _____ Date: _____

Residence: 2836 Rangewood Terrace, Atlanta, Georgia 30345

Post Office Address: 2836 Rangewood Terrace, Atlanta, Georgia 30345

Citizenship: United States

DECLARATION FOR PATENT APPLICATION

(X) Original () Supplemental () Substitute () PCT

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PCT/US99/09151	PCT	27 April 1999	X	

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APPLICATION NUMBER	FILING DATE
60/083,676	30 April 1998

ATTORNEY DOCKET NO. 14114.0325U2**Page 3 of 3**

Full name of third inventor: Leonard W. Mayer

Inventor's signature: _____ Date: _____

Residence: 1703 Little Joe Court, Decatur, Georgia 30033-1519

Post Office Address: 1703 Little Joe Court, Decatur, Georgia 30033-1519

Citizenship: United States

Full name of fourth inventor: Errol Reiss

Inventor's signature: _____ Date: _____

Residence: 3642 Castaway Court, Chamblee, Georgia 30341

Post Office Address: 3642 Castaway Court, Chamblee, Georgia 30341

Citizenship: United States

Full name of fifth inventor: George S. Deepe

Inventor's signature: _____ Date: _____

Residence: 720 Floral Avenue, Terrace Park, Ohio 45174

Post Office Address: 720 Floral Avenue, Terrace Park, Ohio 45174

Citizenship: United States

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ :		A2	(11) International Publication Number:	WO 99/55874
C12N 15/31, C07K 14/37, A61K 39/00, C67K 16/14			(43) International Publication Date:	4 November 1999 (04.11.99)
(21) International Application Number:	PCT/US99/09151		(74) Agents:	SAMPLES, Kenneth, H. et al.; Fitch, Even, Tabin & Flannery, Room 1600, 120 South LaSalle, Chicago, IL 60603 (US).
(22) International Filing Date:	27 April 1999 (27.04.99)		(81) Designated States:	AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
(30) Priority Data:	60/083,676	30 April 1998 (30.04.98)	US	(72) Inventors; and (75) Inventors/Applicants (for US only): ZANCOPE-OLIVEIRA, Rosely, M. [BR/BR]; Rua Fernando Mendes, 25/62, Copacabana, CEP-22021-030 Rio de Janeiro, RJ (BR). LOTT, Timothy, J. [US/US]; 2836 Rangewood Terrace, Atlanta, GA 30345 (US). MAYER, Leonard, W. [US/US]; 1703 Little Joe Court, Decatur, GA 30033-1519 (US). REISS, Errol [US/US]; 3642 Castaway Court, Chamblee, GA 30341 (US). DEEPE, George, S., Jr. [US/US]; 720 Floral Avenue, Terrace Park, OH 45174 (US).
(54) Title:	<u>NUCLEIC ACIDS OF THE M ANTIGEN GENE OF <i>HISTOPLASMA CAPSULATUM</i>, ANTIGENS, VACCINES AND ANTIBODIES, METHODS AND KITS FOR DETECTING HISTOPLASMOSIS</u>			
(57) Abstract	<p>The present invention provides the nucleotide sequence of the M antigen gene of <i>H. capsulatum</i>, which is set forth in the Sequence Listing as SEQ ID NO:1, a nucleic acid having the nucleotide sequence complementary thereto, and nucleic acids having a nucleotide sequence which is substantially the same as the foregoing nucleotide sequences. The present invention also provides vectors and host expression systems containing the foregoing nucleic acids, and isolated or recombinantly-produced antigens encoded by the foregoing nucleic acids. The present invention further provides antibodies generated against the foregoing antigens, and methods and kits for detecting a previous or current <i>Histoplasma capsulatum</i> infection in a subject, and for diagnosing histoplasmosis.</p>			
Published <i>Without international search report and to be republished upon receipt of that report.</i>				

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Page 2 of 3

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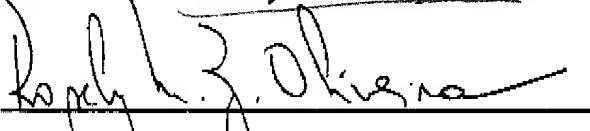
APPLICATION SERIAL NO.	FILING DATE	STATUS (MARK APPROPRIATE COLUMN BELOW)		
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Full name of first inventor:

Rosely M. Zancope-Oliveira

Inventor's signature:



Date: 07.23.2001

Residence:

Rua Fernando Mendes No. 25/62, Copacabana, Rio De Janeiro, RJ 22021-030 Brazil

Post Office Address:

Rua Fernando Mendes No. 25/62, Copacabana, Rio De Janeiro, RJ 22021-030 Brazil

Citizenship:

Brazil



Full name of second inventor:

Timothy J. Lott

Inventor's signature:

Date:

Residence:

2836 Rangewood Terrace, Atlanta, Georgia 30345

Post Office Address:

2836 Rangewood Terrace, Atlanta, Georgia 30345

Citizenship:

United States

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Inventor's signature: _____ Date: _____

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Post Office Address: Rua Fernando Mendes No. 25/62, Copacabana, Rio De Janeiro, RJ 22021-030 Brazil

Citizenship: Brazil

Full name of second inventor: Timothy J. Lott

Inventor's signature: _____ Date: 7/20/01

Residence: 2836 Rangewood Terrace, Atlanta, Georgia 30345

Post Office Address: 2836 Rangewood Terrace, Atlanta, Georgia 30345

Citizenship: United States

Full name of third inventor: Leonard W. Mayer

Inventor's signature: Leonard W. Mayer Date: 27 JUL 01

Residence: 1703 Little Joe Court, Decatur, Georgia 30033-1519

Post Office Address: 1703 Little Joe Court, Decatur, Georgia 30033-1519

Citizenship: United States GA

Full name of fourth inventor: Errol Reiss

Inventor's signature: Errol Reiss Date: 7-20-2001

Residence: 3642 Castaway Court, Chamblee, Georgia 30341

Post Office Address: 3642 Castaway Court, Chamblee, Georgia 30341

Citizenship: United States GA

Full name of fifth inventor: George S. Deepe

Inventor's signature: _____ Date: _____

Residence: 720 Floral Avenue, Terrace Park, Ohio 45174

Post Office Address: 720 Floral Avenue, Terrace Park, Ohio 45174

Citizenship: United States

Full name of third inventor: Leonard W. Mayer

Inventor's signature: _____ Date: _____

Residence: 1703 Little Joe Court, Decatur, Georgia 30033-1519

Post Office Address: 1703 Little Joe Court, Decatur, Georgia 30033-1519

Citizenship: United States

Full name of fourth inventor: Errol Reiss

Inventor's signature: _____ Date: _____

Residence: 3642 Castaway Court, Chamblee, Georgia 30341

Post Office Address: 3642 Castaway Court, Chamblee, Georgia 30341

Citizenship: United States

Full name of fifth inventor: George S. Deepe

Inventor's signature: George S. Deepe

Date: 7/29/01

Residence: 720 Floral Avenue, Terrace Park, Ohio 45174

Post Office Address: 720 Floral Avenue, Terrace Park, Ohio 45174

Citizenship: United States